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THE ORIGIN AND SIGNIFICANCE OF AMMONIA FORMED BY AZOTOBACTER

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INTRODUCTION

Historical

In the voluminous literature which accumulated on Azotobacter during the first quarter of a century after its discovery in 1901, there are to be found only a few sporadic reports which record the occurrence of small amounts of ammonia in pure cultures of the organism. The conditions governing this occasional, almost doubtful, formation of ammonia were never investigated, and a general interpretation of the phenomenon remained in complete obscurity.

Some 10 years ago, however, Kostytschev and his co-workers (18, 19, 20, 22, 23) reported extensive solution culture experiments with *A. "agile"* (17) in which easily measurable quantities of ammonia and amino acids were consistently obtained in the culture medium separated from the cells and pellicles. This work, standing in marked contrast to an enormous number of previous investigations, immediately challenged attention, the more so because apparently no strikingly new cultural methods had been employed. For some time it was believed that the positive results of Kostytschev were explicable mainly upon a basis of strain specificity or culture impurity. In fact, a number of workers of the Russian school, including Demidenko (10), Minenkov (27), and Novogrudsky (28), using *A. chroococcum*, were unable to repeat Kostytschev's experiments. This was true even though additional, very delicate and somewhat novel methods for detecting ammonia were employed.

Eventually Winogradsky (32, 33, 34), using silica gel media, succeeded in obtaining cultures of various strains of Azotobacter which readily produced ammonia when allowed to become alkaline as a result of growing upon alkali salts of organic acids, the latter being preferentially absorbed and leaving a residue of strong alkali. The greatest yields appeared to be obtained in the region of pH 9.0. Comparatively neutral organic salts, such as those of calcium, yielded no ammonia. Small amounts of ammonia were obtained under neutral conditions with cultures grown upon alcohol, but essentially none with mannite or sugar. Other specific, less effective conditions found to favor ammonia liberation by cultures were depletion of substrate; exposure of cultures to vapors of antiseptics such as ether, chloroform, or toluene; emulsification of cells in glycerine; and mechanical destruction of the organisms.

In interpreting his results, Winogradsky concluded that the ammonia liberated by the cells into the culture medium—and, under appropriate conditions, partially distilled spontaneously therefrom—was produced chiefly as a result of a disturbed, delicate balance between two processes: 1, a primary synthesis of N_2 to NH_3 and, 2, an assimilation of this NH_3 in growth and metabolism. His view may be represented schematically in detail as follows:

$$N_2 \text{ (+ dehydrogenase-activated H of organic matter)} \xrightarrow[(N_2\text{-enzyme})]{1} NH_3 \xrightarrow[(\text{growth})]{2} \text{cell nitrogen} \qquad (A)$$

Winogradsky held that steps 1 and 2, although normally in perfect equilibration, were in reality independent, and could be separated under various suitable conditions, the most no-

table and demonstrable being high alkalinity. Any relative retardation of the life processes represented by step 2 would result in the liberation of (unutilizable) NH_3 by way of step 1. It was even suggested, upon the basis of the experiments with antiseptics and cell maceration, that step 1 might proceed in the entire absence of life processes. Autolytic or deaminative formation of ammonia was considered to be rare and negligible, except possibly in extremely old cultures.

Kostytschev had earlier presented much the same scheme or view (19), except for considering, as an additional feature, that step 1 was freely reversible, in accordance with the mass law. He reached this conclusion upon finding that addition of NH_3 to cultures prevented utilization of N_2. He believed that nitrate acted in the same way, although somewhat less effectively per unit of nitrogen, by virtue of being reduced to NH_3; and that peptone also acted likewise, but still less effectively, by virtue of small quantities of NH_3 contained as impurity. He concluded from his experiments, as did Winogradsky later, that NH_3 is the "first" product of N_2-assimilation by Azotobacter.

In his last published paper, based on studies with *A. vinelandii*, Kostytschev (21) arrived at the further modified conclusion that step 2 was to some extent reversible also. His final scheme, worked out with Scheloumova, may be represented as follows:

$$\underset{\text{organic matter}}{N_2 (+ \text{ activated H of})} \underset{3}{\overset{\text{1: primary } (N_2\text{-enzyme})}{\rightleftharpoons}} NH_3 \underset{4\text{: secondary}}{\overset{\text{2 (growth)}}{\rightleftharpoons}} \text{cell nitrogen} \qquad (B)$$
$$\text{(deamination)}$$

Kostytschev and Scheloumova thus held that there were really two important methods of ammonia formation: (primary) direct synthesis from N_2 which occurred only when organic substrate (sugar) was present, and (secondary) deamination of cell nitrogen which occurred only when organic substrate was absent. Under all conditions observed, the secondary formation of ammonia was, in point of fact, greater than the primary, usually by a factor of several fold. In support of the secondary formation, he emphasized especially that no N_2-assimilation took place after the disappearance of the organic substrate; and in support of the primary formation he found that deamination of peptone and glycine took place in the absence, but not in the presence, of organic substrate, from which he concluded that cell nitrogen also would not yield ammonia in the presence of sugar. In this connection, however, Uspensky (30) has since suggested that deamination of non-living material is one thing, whereas splitting off of ammonia from live protein is still another.

Isakova (15) recently reported experiments with both *A. vinelandii* and *A. chroococcum* which confirm those of Kostytschev and Scheloumova (21) in showing the production of ammonia in neutral or only very slightly alkaline media containing mannite or glucose, as well as salts of organic acids. In the light of these results, Winogradsky's view as to the relative importance of extreme alkalinity in ammonia formation appeared to require considerable modification.

The recent note of Bach, Yermolieva, and Stepanian (2), reporting the production of NH_3 from N_2 and glucose by "non-living," cell-free press juice extracts of Azotobacter cells, and awaiting extension and confirmation, is for the present beyond the immediate considerations of this paper, which deals with ammonia formed by or from Azotobacter cells; eventually a close relationship between the two investigations might develop.

Critique of previous investigations

The investigations just described evidently possess an important agronomic significance, in demonstrating the potentially immediate, fertilizing rôle of Azotobacter, long held highly questionable because of the very slow decomposition of these organisms by well-known microbiological agents.

A second, equally important aspect of the observed evolution of ammonia by cells of Azotobacter is its possible relation to the chemical mechanism of nitrogen fixation by these organisms: Is, or is not, ammonia a major, essential intermediate product formed in the complicated process whereby gaseous N_2 is converted into organized cell nitrogen? Unfortunately, for a number of reasons, the experimental findings cited, although at first sight suggestive in this connection, cannot be said to provide any conclusive answer to this difficult question. It will be very desirable at this point to indicate some of the more immediately evident criticisms; others will be postponed until discussion of the experimental results presented in this paper. The chief inherent weaknesses of the earlier investigations may be said, in general, to lie in their essentially qualitative, rather than quantitative outlook, and in their failure to include certain clearly necessary control experiments, and certain very desirable types of related experiments to be mentioned shortly.

In the previous investigations the cultures were at no time placed in an atmosphere devoid of nitrogen gas, in order to determine the necessity for the presence of N_2 for NH_3 formation, that is to say, for the actual simultaneity of NH_3-formation with N_2-disappearance. Nor were studies with various qualitative and quantitative mixtures of oxygen, nitrogen, and inert gases (i.e., H_2) carried out to determine the various influences of such gases.

It was not shown that cultures grown in a considerable variety of fixed nitrogen compounds did not likewise give off NH_3, and thus establish a reasonable specificity for the evolution of NH_3 from cultures grown with free N_2. Kostytschev performed a few such experiments with nitrate, which will be considered later.

The capacity of mature cultures, grown in either free or fixed nitrogen, to ammonify a wide variety of added, known fixed nitrogen compounds, particularly those types of probable occurrence in Azotobacter cells, was not determined to any extent.

The course of sugar consumption was not followed quantitatively in relation to ammonia formation, nor was respiration—oxygen consumption or carbon dioxide production—studied. The relative importance of these probably very important factors was thus impossible to assess.

Finally, the technique employed was in general inadequate to determine reasonably closely the optimum conditions for ammonia production, with respect to important variables such as pH, temperature, anaerobiosis, added organic matter, cell concentration, and inhibiting agents. The chief reasons for this lay in the heterogeneity of the solid, or pellicle-liquid, stagnant cultures employed, involving diffusion limitation, and non-uniformity and uncertainty of concentration of various nutrients, from one cell to another in a given culture. It was therefore relatively difficult either to impose or to measure, with desirable accuracy, the environmental conditions, which varied in different regions of one and the same culture. The subtle bearing of this circumstance should be fully appreciated when interpreting findings heretofore obtained.

Statement of problem

In this and two accompanying papers (7, 14) are reported, with due appreciation of the criticisms just advanced, the results of a detailed and widely varied investigation directed in the interest both of obtaining many further details of agronomic import concerning the formation of ammonia by Azotobacter and with a view to ascertaining, so far as possible, whether the ammonia observed is liberated *before* a synthesis into normal cell nitrogen, as in schemes A

or B of Winogradsky and Kostytschev, or *after* a synthesis into normal cell nitrogen. Is none, some, or all of the extracellular NH_3 *which has been observed* derived from cell nitrogen, or to what extent is it derived specifically, uniquely, and directly from N_2?

EXPERIMENTAL

General methods

Cultures and media. The methods of culture, and the Warburg technique used to measure oxygen consumption, carbon dioxide production, and respiratory quotient, have been previously described in considerable detail (3, 5, 9, 13, 25). The "stock cultures," used for purposes of inoculation, or in the preparation of "stock suspensions," have been grown in an air thermostat at 28°C. in either 250-cc. gas wash bottles containing 100 cc. of (sterile) culture medium, or 2.5-liter reagent bottles containing 2 liters of medium, aerated with air (or occasionally 50 per cent O_2 in N_2) at the rate of 25 to 200 cc. per minute. The culture medium consisted of 1 to 2 per cent glucose (or sucrose) and 0.00025 gm. $Na_2MoO_4 \cdot 2H_2O$ and 0.005 gm. synthetic (sugar) humate containing 0.0005 gm. Fe, made up in the clear, liquid "inorganic medium" obtained after the following mixture had been thoroughly shaken, allowed to stand, and settle: 0.8 gm. K_2HPO_4, 0.2 gm. KH_2PO_4 (pH 7.2), 0.2 gm. $MgSO_4 \cdot 7H_2O$, 0.2 gm. NaCl, 0.1 gm. $CaSO_4 \cdot 2H_2O$, and 1000 gm. H_2O. The clear solution contains about 15 per cent less phosphate and 40 per cent less calcium than the unsettled liquid. Unless otherwise indicated, this "inorganic medium" was employed in making up all cultures, sometimes being fortified, for greater buffer action, with addition of 3 to 10 times the normal phosphate mixture of the desired pH.

The "stock suspensions" were prepared by centrifuging the "stock cultures" under refrigeration in 500-cc. bottles at 2000 r.p.m., or occasionally in the Sharples supercentrifuge. The material was then washed and centrifuged twice with inorganic medium diluted fivefold with distilled water, and finally taken up in this same diluted medium at a concentration 10 to 100 times the original cell concentration, generally 5 to 25 billion per cubic centimeter (about 4 to 20 mgm. cell dry matter per cubic centimeter, or 1 to 5 mgm. cell N per cubic centimeter (table 10)). These suspensions were stored at 5°C. until needed and would remain essentially unchanged for over a month, with negligible NH_3-formation and no decrease in respiration capacity (tables 10 and 11). For use in the Warburg vessels the stock suspensions of organisms were diluted two- to tenfold with inorganic medium diluted fivefold with distilled water, but fortified with 3 to 10 gm./l. of phosphate buffer. Apart from concentrating the material and renewing the inorganic medium, no effect, physical, chemical, or physiological, ascribable to centrifuging carried out as indicated, has ever been observed concerning the behavior of the cells (table 9). No significant growth or contamination was ever observed to develop in these suspensions while maintained at low temperature and containing neither fixed nitrogen nor organic substrate.

As gas phases, there were employed, in addition to air, pure oxygen and various (nitrogen-free) mixtures of O_2 in H_2, prepared by means of a multiple flowmeter (3); and also, to provide for anaerobic conditions, pure H_2 or pure N_2 passed through alkaline pyrogallol to eliminate traces of O_2. These various gases and mixtures were employed with both cultures and suspensions in both Erlenmeyer and Warburg microrespiration flasks.

The respiration rates in the Warburg apparatus were usually measured hourly for the first 1 to 6 hours, then possibly overnight, if the magnitude permitted, otherwise the manometers were left open to the air. The same procedure was followed on second, third, and later days, when involved. The Q_{O_2}, or cubic millimeters O_2 consumed per milligram of cell dry matter per hour, was usually calculated from readings several times larger than its own magnitude (several milligrams dry matter being involved). Various values reported, particularly in tables 10 and 11, permit an estimate of the usual accuracy attained.

Three strains of Azotobacter were studied: *A. vinelandii*, a vigorous nitrogen-fixing organism, obtained originally from the New Jersey Agricultural Experiment Station and used in this laboratory for many years; *A. chroococcum*, a stock culture obtained through the courtesy of N. R. Smith of the Bureau of Plant Industry; and *A. chroococcum* Strain S.M. 1, obtained in 1927 from the Rothamsted Experimental Station and isolated from a Harpenden soil.

Analytical methods. The pH was determined either colorimetrically, with one or more La Motte standards, or electrometrically, with a Welch quinhydrone apparatus.

Glucose was determined in some cases by the well-known Bertrand, copper reduction method, which is not affected by the presence of Azotobacter cells. Ordinarily, however, glucose was determined by a method worked out using Nessler's reagent. At concentrations greater than 0.001 to 0.005 per cent glucose, this reagent gives a red precipitate, which is photolabile and will turn yellow or black as the surrounding light intensity is greatly increased. The precipitate is given also by fructose, maltose, lactose, l-arabinose, and l-xylose, but not to any extent by sucrose, mannite, succinate, lactate, benzoate, acetate, or glycerophosphate. There is normally an induction period of at least 5 to 20 seconds, depending upon the concentration, before any precipitate develops at all, and 15 to 45 minutes are required for complete precipitation. Azotobacter cells have never been observed to give any reaction themselves but, when present in high concentration, may appear to hasten the reaction given by glucose, and in this case it is preferable to have the standards contain (glucose- and NH_3-free) cells also. In making the test 0.1 to 0.3 cc. of unknown are added to 1 cc. of Nessler's reagent prepared by the method of Arny and Ring (1), and compared against standards. This convenient method of determining the order of concentration of glucose (etc.) has not, to our knowledge, been reported hitherto.

NH_3-N was determined by microdistillation or by Nesslerization, with concordant results if both methods were used (tables 7 and 16). The Nesslerization was carried out with 0.1 to 0.3 cc. unknown added to 1 to 2 cc. reagent, with cells in the standards where very high concentrations produced shading. The unknowns were always diluted to contain 10 mgm./l. NH_3-N or less, so as to obtain color changes only, and no precipitation. The reaction product was not photolabile, and was formed in a few seconds, so that normally the subsequent formation of precipitate by any glucose present did not interfere; nor, conversely, did NH_3-N affect the glucose test, which was normally one or more orders more intense. The NH_3-N determinations were usually accurate to ± 5 to 10 per cent, with ± 25 per cent as an occasional maximum. The relative order of accuracy was arbitrarily varied in accordance with the relative importance of a given experiment. Needless to say, where necessary, the determinations were corrected for blank and control values. The NH_3-N concentrations in undiluted cultures ranged, in absolute magnitude, up to 500 mgm./l., so that with determinations at 1 to 5 mgm./l an accuracy of about 1 per cent was attained for the order involved. Microdistillation provided a somewhat greater relative accuracy than Nesslerization, and was carried out for 5 to 10 minutes in a modified Pregl apparatus. The cells themselves did not decompose to provide NH_3-N under these conditions. In several hundred distillations performed during the past 7 years on cultures which had been grown in either N_2-N, nitrate-N, or NH_3-N, for 1 to 180 days, no traces of NH_3-N (0.2 mgm./l. or less) were ever obtained, even after distillations of from 10 minutes to 2 hours, provided there was no NH_3-N in the medium surrounding the cells, as indicated by negative Nessler's test carried out at ordinary temperatures.

Growth was measured in four ways. Total nitrogen was determined with the macro-Kjeldahl method, carried out by Mrs. E. Rist, or with the micro-Kjeldahl method, carried out by Dr. R. T. Milner and Mrs. M. S. Sherman, of the Analytical Division of this Laboratory. Cell-dry matter was determined by centrifuging aliquot portions of culture in weighed cups, discarding the clear liquid, drying at 100°C., and weighing. Turbidimetric measurements with arbitrary standards were made visually or turbidimetrically with a Bausch and Lomb nephelometer. Cell counts were made with specially constructed bacterial haemacytometers of 0.002 mm. depth.

Preliminary studies

Confirmation of previous work. Our first efforts were directed toward repeating the previously published work. As a variant in procedure, it was found very simple and convenient to grow cultures in shallow layers of liquid in Erlenmeyer or Roux flasks, with occasional shaking by hand to prevent pellicle formation, and to maintain a reasonably uniform distribution of cells. A general confirmation of the earlier, positive experimental findings was easily obtained with this technique. Cultures growing in common inorganic media containing *initially* 0.1 to 3 per cent organic substrate readily yielded ammonia after varying periods of incubation under appropriate conditions. The organic substrates studied were glucose, sucrose, and the sodium salts: succinate, benzoate, acetate, butyrate, lactate, and malonate. In several instances the yields of NH_3-N obtained were 30 to 40 per cent of the total cell nitrogen involved and were thus somewhat greater than any reported previously. The actual concentrations of NH_3-N found in the medium usually ranged up to 10 mgm./l. (10 p.p.m.), but in some cases attained 30 to 40 mgm./l., even in essentially open (cotton-stoppered) flasks, where loss by evaporation often amounted to 10 to 50 per cent.

The best results were obtained with initial mixtures of 0.5 to 2 per cent glucose and 0.2 to 0.5 per cent organic alkali salt. The sugar provided a greater increase in cell mass (growth), and the organic salt a more favorable, alkaline reaction. With sufficient organic alkali salt, the pH rose rapidly to 9 and, with an excess, attained at times a value of 11, even in the presence of the carbon dioxide of the air. Little if any growth or nitrogen fixation was observed to take place above pH 9.0 to 9.3, or after the formation of NH_3 had commenced, or, in accordance with the findings of Kostytschev and Scheloumova (21), after the disappearance of the organic substrate.

Tables 1 to 8 are illustrative of the methods and results obtained in 73 out of a total of over 300 cultures involved in the preliminary study. Because of their exemplary or confirmatory nature, and because the later, more refined work will bring out all the new major points more clearly, these tables will not be analyzed individually except for the notes appended to them. However, the following general variables of procedure or situation may be noted in particular: sources and initial concentrations of organic substrates (tables 1, 2, 4); residual concentration of glucose (tables 3, 4, 5); nitrogen substrates for growth (tables 2, 3); pH (table 7); residual (essentially cell-free) medium (table 6); marked evaporation of NH_3-N (tables 2, 6, 7, 8) and method of stoppering (table 1); relative and absolute volumes of culture media and flasks (tables 2, 4); size and kind of inoculum, including species; duration of incubation and onset of NH_3-formation, course of NH_3-formation and pH change; temperature.

New findings. In addition to confirmatory features, several new findings obtained in the preliminary study, besides the increased yields of NH_3-N already noted, should be observed. The replacement of 78 per cent N_2 in O_2

by 78 per cent H_2 in O_2, without alteration in NH_3-formation, qualitatively or quantitatively (table 1), is highly indicative of the non-existence of an immediate relationship between NH_3-formation and N_2-disappearance, and has a marked negative bearing in connection with schemes A and B. The equivalent replacement of N_2 by nitrate-N, as a source of nitrogen for growth and NH_3-

TABLE 1

Influence of glucose, concentration of succinate, and hydrogen-oxygen gas on the course and extent of NH_3-formation

INITIAL SUBSTRATE		DAYS						
		0	1	2	3	4		5
		Gas phase						
		Air	Air	Air	Air	Air	21 per cent O_2 in H_2 from 3rd day*	Air
A. 1 per cent glucose	pH	7.0	7.0	7.0	7.0	7.0	7.0	7.0
	NH_3-N	0	0	0	0	0	0	0
B. 2 per cent Na-succinate	pH	7.0	7.3	7.8	9.0	9.3	...	...
	NH_3-N	0	0	0	0	0	...	0
C. 0.7 per cent Na-succinate	pH	7.0	7.4	8.2	8.8	9.3	9.3	...
	NH_3-N	0	0	0	1.4	26	27	40
	Total-N					127	128	(127)
	$\frac{NH_3\text{-N}}{\text{Total-N}}$, per cent					21	21	32

25 cc. inorganic culture medium in 750-cc. closed (rubber-stoppered) Erlenmeyer flasks, inoculated with 5 cc. of 6-day-old, aerated stock *A. vinelandii* culture in which original glucose was nearly consumed. Temperature, 34°C. NH_3-N (microdistillation) and total-N (Kjeldahl) in mgm./l.; pH colorimetric.

* A and C cultures each divided on third day (when NH_3-formation was just commencing in C culture) into two aliquots then placed in air and in 21 per cent O_2 in H_2, respectively.

Notes: The absence of NH_3 in the 2 per cent succinate culture as compared with the 0.7 per cent succinate culture, *in spite of similar courses of pH change*, is ascribable to substrate presence in the former even after 5 days, but its disappearance by 3 days in the latter. Presence of substrate, and to some extent low pH, prevented NH_3-formation in the 1 per cent glucose culture. Growth was heavy in all three cultures, but somewhat differentiated in the decreasing order, 1 per cent glucose, 0.7 per cent succinate, 2 per cent succinate. The replacement of N_2 by H_2 in the (21 per cent O_2) gas phase has effected no change in the extent or course of NH_3-formation in either the glucose or 0.7 per cent succinate cultures.

formation (table 2), has a similar significance. Both these findings will be still further enlarged and generalized, but it is to be noted here that in the particular experiments on which these findings are based NH_3-formation was actually rather large; consequently their significance and relation to previously published work is in any case assured.

TABLE 2

Influence of source of nitrogen for growth, and succinate concentration, on NH_3-formation

N-SOURCE FOR GROWTH	CULTURE	FLASK	SUCCINATE		DAYS						
	cc.	*cc.*	*per cent* Na-salt		0	2	3	4	6	7	12
N_2-N	6	150	1.5	pH	7.0	9.31	9.5	9.73	9.8	10.0	....
				NH_3-N	0	0	0.2	0.4	0.4	....	....
			1.0	pH	7.0	9.14	9.4	....	9.8	....	10.0
				NH_3-N	0	1	1.5	2	0.8	....	....
			0.5	pH	7.0	9.05	9.3	9.22	9.5	....	9.8
				NH_3-N	0	4	6	5	1.5	....	....
			0.25	pH	7.0	8.85	9.0	8.97	8.6	....	9.2
				NH_3-N	0	2	6	5	2.5	....	....
Nitrate-N 200 mgm./l.	6	150	1.5	pH	7.0	9.41	9.6	....	9.7	....	10.1
				NH_3-N	0	0	1	1	0.4	....	....
			1.0	pH	7.0	9.41	9.4	....	9.8	9.9	....
				NH_3-N	0	1.5	1	1	1.5	....	....
			0.5	pH	7.0	9.14	9.4	....	9.7	....	....
				NH_3-N	0	4	7	5	2.5	....	....
			0.25	pH	7.0	8.88	9.2	....	9.4	....	9.6
				NH_3-N	0	3.5	6	6	4	....	....
N_2-N	10	500	1.5	pH	7.0	9.65	9.6	9.56	9.8	....	9.9
				NH_3-N	0	0	0.5	0.5	0.7	....	....
			1.0	pH	7.0	9.31	9.5	....	9.6	....	10.1
				NH_3-N	0	1	1	0.5	0.4	....	....
			0.5	pH	7.0	9.22	9.3	....	9.7	10.0	....
				NH_3-N	0	3.5	4	1.5	1.5	....	....
Nitrate-N 200 mgm./l.	10	500	1.0	pH	7.0	9.56	9.5	9.59	9.7	10.0	....
				NH_3-N	0	1.5	1	0.7	0.5	....	....

1-day old, aerated stock *A. vinelandii* culture, growing in 1 per cent glucose medium, placed in cotton-stoppered flasks with volumes as indicated. Temperature, 32°C. Gas phase, air. NH_3-N (Nesslerization) in mgm./l. Total cell-N (not determined) estimated never to have exceeded 100 mgm./l. pH by quinhydrone electrode (2, 4 days) and colorimetrically (3, 6, 7, 12 days).

Notes: The optimum NH_3-formation at about 0.5 per cent Na-succinate represented, for the most part, a balance between greater growth but slower completed substrate utilization with increasing succinate concentration. Evaporation of NH_3 from the (cotton-stoppered) flasks played a certain rôle in determining the absolute concentrations of NH_3 observed in the liquid medium. The rapid increase to pH 9 by 2 days is to be noted, and also its continued more gradual rise to a value of 10 after a week or more. The pH change was slightly greater in the larger flasks; the converse was true, however, of NH_3-formation, probably because of the actual high pH range involved. The pH increases regularly with increasing initial succinate concentration. The cultures obtaining their nitrogen from nitrate gave results in regard to pH change and NH_3-formation essentially indistinguishable from cultures obtaining their nitrogen for growth from free N_2. Similar cultures grown in urea, peptone, or asparagin gave NH_3 in the same manner; and also to a certain extent, independently of any growth taking place, by direct deamination of these compounds.

As the predominant new observation, it was established, as an invariable rule, that the formation of NH_3 never commenced until the concentration of utilizable organic substrate had reached a very low level of about 0.03 to 0.01 per cent (tables 3, 4, 5, 6) and had thus essentially disappeared. It became quite clear that the question of whether appreciable amounts of NH_3-N would appear at all was determined almost wholly by this one factor of concentration of utilizable organic substrate, independently of any other external factor such as pH or temperature varied over a wide range. For various reasons, indicated in table 6, the last traces of glucose disappear very slowly in

TABLE 3

Influence of residual glucose on NH_3-formation by cultures grown with both N_2 and NH_3

	11 DAYS			16 DAYS		
Initial NH_3-N, added at 0 days (mgm./l.)	20.0	40.0	60.0	20.0	40.0	60.0
Total-N, mgm./l.	51.2	64.1	78.5	59.7	66.6	84.0
N fixed, mgm./l.	31.2	24.1	18.5	39.7	26.6	24.0
NH_3-N, mgm./l.	0.0	0.0	0.0	0.0	0.0	2.3
Glucose conc., per cent	0.365	0.312	0.276	0.015	0.015	0.000
Cell dry matter (mgm./l.)	468	551	733	551	545	649
N in dry matter, per cent	10.9	11.6	10.7	10.8	12.2	12.6
pH	7.2	7.2	7.2	7.2	7.2	7.2

100 cc. of 1 per cent glucose medium in cotton-stoppered, stagnant, 1000-cc. (flat) Roux bottles inoculated with 6 drops of 6-day-old, stagnant culture of *A. chroococcum* Strain S.M. 1. Temperature, 28°C. Gas phase, air. Initial pH 7.2 ±0.1. All cultures and analyses performed in duplicate, and averaged. NH_3-N determined by microdistillation (sensitive here to 0.2 mgm./l.), total-N by Kjeldahl, and glucose by Bertrand reducing method (sensitive to 0.002 per cent).

Notes: It is to be seen that by 11 days the glucose was two-thirds consumed, and all added NH_3 had been utilized, with additional N_2 fixed, the moreso the smaller initial NH_3-addition. By 16 days smaller additional amounts of nitrogen had been fixed, and the glucose had in two instances almost but not quite disappeared, whereas in the third instance it had completely disappeared, and there was a definite loss in cell dry matter (presumably through respiration), and NH_3-formation had clearly commenced, even at the low pH value of 7.2 involved. The percentage nitrogen in the cell dry matter remained fairly constant in spite of widely varying nitrogen nutrition, and a certain variation in cell dry matter obtained.

stagnant cultures (tables 3, 4, 5, 6) and there is a period of one or more days when NH_3-N may form with small amounts of sugar still present, as in Kostytschev and Scheloumova's experiments (21), as well as in ours. Kostytschev and Scheloumova did not report any analyses of concentrations of residual sugar, and so missed the significance of the concentration factor emphasized here. Indeed, the use of solid media in their experiments prevented in considerable measure any satisfactory determination of substrate concentration effects. In the same way, Winogradsky had observed that NH_3-formation commenced, in cultures with alcohol as organic substrate, after disappearance

of the odor of the alcohol, and then rather suddenly; and in cultures containing both mannite and Na-lactate, after browning of the mucus, an indication of disappearing substrate. Thus, although depletion of organic substrate

TABLE 4

Influence of various initial mixtures of glucose and succinate on NH_3-formation

SUBSTRATE MIXTURE		DAYS											
Succinate	Glucose	1		2			3		4			7	
per cent Na-salt	*per cent*	pH	NH_3-N	pH	NH_3-N	Glucose	pH	NH_3-N	pH	NH_3-N	Glucose	pH	NH_3-N
0	0.1	7.6	0	7.8	0	0.05–0.1	7.7	0	8.1	0	0–0.03	8.3	1.8
	0.2	7.6	0	7.9	0	0.05–0.1	7.7	0.7	8.1	3.5	0–0.03	8.3	3.5
	0.4	7.4	0	7.6	0	0.05–0.1	7.8	0	8.1	1.5	0–0.03	8.1	4.5
	1	7.3	0	7.2	0	0.05–0.1	7.6	3.5	7.9	6.5	0–0.03	7.9	8.5
0.1	0.1	7.6	0	8.5	0	0.05–0.1	8.9	0	8.8	0	0–0.03	8.9	0.8
	0.2	8.0	0	8.4	0	0.05–0.1	8.8	0	8.7	1.5	0–0.03	9.0	1.5
	0.4	7.6	0	8.4	0	0.05–0.1	8.6	2.5	8.8	1.5	0–0.03	8.9	1.8
	1	8.0	0	7.4	0	0.05–0.1	8.5	4.5	8.5	6.5	0–0.03	8.9	3.5
0.3	0.1	8.3	0	8.8	0	0.05–0.1	9.2	0.4	9.2	0.4	0–0.03	9.3	0.3
	0.2	8.4	0	8.6	0	0.05–0.1	9.0	0.3	9.3	0.7	0–0.03	9.4	0.7
	0.4	7.7	0	8.2	0	0.05–0.1	9.1	1.5	9.4	1.5	0–0.03	9.4	1.2
	1	7.7	0	7.7	0	0.05–0.1	8.2	0.8	9.1	5.5	0–0.03	9.3	3.5
0.3	0.1	8.4	0	8.8	0	0.05–0.1	9.2	2.2	9.3	1.5	0–0.03	9.1	0.3
(6 cc. per	0.2	8.3	0	8.9	0	0.05–0.1	9.3	2.2	9.2	2.5	0–0.03	9.0	1.3
150-cc.	0.4	8.2	0	8.4	0	0.05–0.1	8.8	0.3	9.2	0.2	0–0.03	9.1	1.5
flask)	1	8.2	0	7.8	0	0.05–0.1	8.5	0	8.7	0	0.05–0.1	9.0	0.7
1	0.1	8.3	0	9.2	0	0.05–0.1	9.6	0	9.7	0	0–0.03	9.9	0
	0.2	8.4	0	9.0	0	0.05–0.1	9.6	0	9.7	0	0.05–0.1	9.8	0.3
	0.4	8.2	0	9.1	0	0.05–0.1	9.6	0	9.6	0	0.05–0.1	9.8	0.3
	1	8.4	0	9.0	0	0.05–0.1	9.5	0	9.6	0	0.05–0.1	9.6	0

500-cc. Erlenmeyer flasks (cotton-stoppered) with 10 cc. of 3-day-old, aerated stock *A. vinelandii* culture diluted fivefold with inorganic medium (pH 8) in order to reduce initial glucose concentration to less than 0.2 per cent. Temperature, 32°C. Gas phase, air. NH_3-N in mgm./l., glucose in per cent.

Notes: The concentrations of NH_3-N formed depend upon concentration and kind of substrate, duration, (unmeasured) growth, and evaporation of NH_3. The pH increases regularly with succinate concentration (as in table 2), and decreases with glucose concentration.

was recognized as a factor in NH_3-formation, it was not assigned the supreme importance warranted and was considered minor compared to pH and other factors.

Practically no measurements are given, in tables 1 to 8, of the amount of growth involved in any case. It may be said, however, that for NH_3-formation the factor of cell mass (cell nitrogen, or cell number, or turbidity) was next in importance to substrate concentration and, in general, of at least equal importance to pH and (where allowed to take place) physical evaporation. Because of the fact that NH_3-N never exceeded as a limit about half the cell nitrogen, the amount of NH_3-N formed was, in order of magnitude, closely related to

TABLE 5

NH_3-content of miscellaneous (not strongly alkaline) stock cultures, in relation to glucose content and age

AGE	pH	GLUCOSE	NH_3-N
days		*per cent*	*mgm./l.*
54	7.5	0	55
46	7.8	0	55
56	7.2	0	18
56*	7.0	0.01	7
29	7.2	0	6
39	7.3	0.01	5
55*	6.9	0.01	4
15*	6.6	0.01	3
33*	6.6	0.05	0
29*	6.6	0.05	0
39*	6.8	0.08	0
16	7.0	0.05	0
0–10	6.0–8.0	0.05–1.0	0†

Stock *A. vinelandii* cultures grown on 100 cc. 1 to 2 per cent glucose or sucrose medium in 500-cc. gas wash bottles aerated slowly with sterile air.

* Molybdenum content of medium partially deficient and growth reduced one-half to three-quarters its value with adequate Mo.

† Numerous (*A. vinelandii* or *A. chroococcum*) stock cultures 0 to 10 days old, and still containing 0.05 to 0.10 or more per cent glucose (and varying in pH from 6.0 to 8.0), practically never gave even traces of NH_3 (less than 0.1 mgm./l.). This was equally true, of course, for many similar cultures up to at least 90 days old.

Notes: The concentration of available glucose completely determines the qualitative appearance of NH_3-N; and pH and extent of growth (as varied by age and molybdenum content) are additional, secondary quantitative factors.

the mass of organisms. Any conditions which decreased the extent of growth before the substrate had disappeared, although otherwise favorable to NH_3-formation, resulted in smaller eventual yields of NH_3.

Tables 1 to 8 illustrate well the complexity and tangling of factors necessarily involved in the sort of technique employed heretofore and in these preliminary studies. In general, satisfactory interpretation of the tables already given requires simultaneous consideration of all the factors indicated in this section; it was, nevertheless, rarely feasible or convenient to carry out all the necessary measurements regarding them. It very soon became evident,

TABLE 6

Influence of residual glucose concentration and clear centrifuged, mature culture medium on NH_3-formation

CULTURE A*	SUSPENSION B*	RESIDUE C*	INORGANIC MEDIUM*	ADDITIONAL		DAYS				
cc.	cc.	cc.	cc.	0.4 per cent		2	3	4	6	8
10					pH	7.4	7.9	8.0	7.9	8.6
					NH_3-N	0	0	0	0	7.5
					Glucose	...	0.10	0.07	0.04	0.01
	10				pH	7.8	7.9	7.9	8.3	7.8
					NH_3-N	0	0.5	1.7	27	6.5
					Glucose	...	0	0	0	0
	5	5			pH	7.4	8.0	8.1	8.2	8.3
					NH_3-N	0	0	0	7.5	14
					Glucose	...	0.1	0.03	0.01	0
	5	2	3		pH	7.7	7.9	7.8	8.3	8.0
					NH_3-N	0	0	0	11	6.5
					Glucose	...	0.03	0.03	0.01	0
	5	0.5	4.5		pH	7.8	7.7	7.8	8.1	8.0
					NH_3-N	0	0	0.4	14	2.5
					Glucose	...	0	0	0	0
	5	2	3	Na-succinate	pH	8.5	9.2	9.4	9.5	9.6
					NH_3-N	0	3.5	4.5	3.5	1
					Glucose	...	0.01	0.01	0.01	0
	5	5		Glucose	pH	7.4	7.6	8.0	8.3	8.3
					NH_3-N	0	0	0.5	22	17
					Glucose	...	0.05	0.01	0	0

* 4-day-old, aerated stock *A. vinelandii* culture A centrifuged; clear liquid residue C retained; solid cell residue taken up in 0.1 per cent glucose, inorganic medium at pH 8 at two and a half times original cell concentration in culture A to yield suspension B. These mixed in varying proportions as indicated, using 10 cc. total volume per 500-cc. cotton-stoppered Erlenmeyer flasks. Temperature, 32°C. Gas phase, air. NH_3-N (Nesslerization) as mgm./l.; glucose as per cent. 100 mgm./l. initial total cell nitrogen in culture A, 250 mgm./l. in suspension B.

Notes: The evaporation of NH_3-N is to be noted as rather marked between the sixth and eighth day; the rather slow disappearance of the last traces of sugar is probably due to a very low respiration capacity on the part of the cells, and may also be sustained in part by reconversion from small amounts of reserve higher carbohydrate. The low concentration of the sugar also provides for only a small saturation of the respiration enzyme system of the cells. The increased NH_3-formation due to added glucose is a result of increased cell nitrogen (growth), and this is also true for the case of 10 cc. of suspension B compared to 10 cc. of culture A, where two and a half times as much cell nitrogen (Kjeldahl) is involved. In this experiment the NH_3-formation has depended chiefly upon available cell nitrogen and residual glucose concentration and scarcely at all upon pH. No clear-cut effect of centrifuged liquid residue, markedly hastening or retarding, is evident. The pH of 9 or more attained with the 0.4 per cent additional succinate has clearly been detrimental.

TABLE 7

Influence of pH upon total NH_3-formation and NH_3-evaporation

		Initial pH..... Final pH.....	7.2 7.5	8.0 8.1	8.7 8.4
NH_3-N	Initial	Nesslerization	0	0	0
	Final	Nesslerization	40	32	23
		Microdistillation	36	32	26
Total-N	Initial	Kjeldahl	125	125	125
	Final		116	...	98
NH_3-N	Lost	(Evaporation)	9	...	27
NH_3-N	Total	(Final + Lost)	47	...	52
$\frac{\text{Total } NH_3\text{-N}}{\text{Initial Total-N}}$, per cent			37	...	42

13-day-old, aerated stock *A. vinelandii* culture centrifuged and taken up in inorganic, borate-phosphate medium at three different pH values, with 6 cc. per 150-cc. cotton-stoppered flask. Temperature, 32°C. Gas phase, air. NH_3-N and total-N in mgm./l. Duration, 5 days.

Notes: The final amounts of NH_3-N obtained do not vary greatly, over the pH range studied. Other similar experiments not reported, in which the course of NH_3-formation was followed, after initial adjustment of pH, indicate an optimum rate at about pH 8, with greatly reduced rates below pH 7 and above pH 9.

TABLE 8

Rate of evaporation of NH_3 from culture medium, in relation to pH

	DAYS			
	0	1	2	4
pH	8.9	8.2	8.0	7.9
Conc. NH_3-N (mgm./l.)	10	5.5	3	3
	3	1.5	0.6	0.6
	1.5	0.7	0.5	0.5
	0.5	0.2	0.1	0.1
pH	7.3	7.2	7.2	7.2
Conc. NH_3-N (mgm./l.)	10	9.5	9.5	9.5
	3	3	2.7	2.7
	1.5	1.5	1.4	1.4
	0.5	0.5	0.5	0.5

10 cc. uninoculated, inorganic (sugar-free) medium in 500-cc. cotton-stoppered Erlenmeyer flasks. Temperature, 32°C. Gas phase, air.

Notes: Up to an initial concentration of 10 mgm./l. NH_3-N, the factor of evaporation of NH_3 becomes significant only above pH 8. The pH was independent of the NH_3-N concentration and altered on account of the CO_2 in the air. In other experiments at concentrations of 25 to 50 mgm./l. NH_3-N evaporation was appreciable below pH 8.

particularly in the case of the three major factors involved—carbohydrate concentration, cell mass, and pH—that no one factor could, by the technique employed, be controlled independently or even made to act uniformly in the same direction. Thus, the use of glucose increased cell mass favorably but tended, unfavorably, to keep the pH too low. Use of organic alkali salts increased the pH favorably up to a certain point, beyond which, however, cell mass, respiration, and NH_3-formation became very small; loss by evaporation increased continuously with pH and also with extent of NH_3-formation. If cell mass were increased, as by centrifugation, the carbohydrate concentration was more quickly lowered, favorably, but the pH was also lowered, unfavorably, as a result of the increase in respired carbon dioxide. It was, consequently, extremely difficult to obtain reasonably satisfactory functional relationships between NH_3-formation and any one independently varied major factor. For example, the optimum pH of about 9 assigned by Winogradsky is only apparent, being, in fact, about a unit too high (fig. 3). The value of 9 was observed in cultures in which the organic alkali substrate had essentially disappeared, either locally or throughout the solid medium, by which time the pH had already, under the particular arrangement of experimental conditions involved, happened to have attained a value actually greater than true optimum; the pH had appeared to be optimum simply because the still more important (insufficiently appreciated) factor of organic substrate concentration was dominating the situation.

Aerobic and anaerobic formation of NH_3 under optimum conditions

General technique. As a result of the preliminary experiments just described, in which it was found that low concentration of organic substrate and large cell mass warranted major attention in observing NH_3-formation, it was decided to use centrifuged, washed cultures to provide a large concentration of cells and ready elimination of organic substrate, rather than attempt to obtain the same ends by several days' growth and substrate consumption. Such suspensions could easily be adjusted to the optimum pH of 7.8 to 8.0. With this systematic, more controllable technique it was further desirable and convenient to employ the Warburg micro-respiration apparatus, with its manifold variety of possible experimentation.

It first became necessary to determine whether centrifuging produced any unanticipated effects. All our past experience had provided no support for this possibility when the centrifuged cells were tested in other connections, such as growth, nitrogen fixation, or respiration; and Azotobacter cells appeared to be comparatively stable with respect to physical treatment, such as mechanical grinding or drying. This view receives further confirmation in the results presented in table 9. This table indicates that cultures of widely varying age suffer no significant loss of soluble nitrogen compounds due to the processes of centrifuging, washing, and storing (with refrigeration) employed by us. This is true even if distilled water is used instead of inorganic nutrient medium in carrying out the washings. The pH and glucose contents of solution cultures remain essentially unchanged by centrifuging and washing, and the celite used in filtering held back no ammonia and only a small fraction of the soluble nitrogen compounds. It will be recalled also that in table 6 the centrifugate likewise appeared to contain no material noticeably harmful or beneficial to NH_3-formation. It is to be noted in table 9 that no NH_3-formation took place in the stock cultures 1 to 4 days old, where sugar still remained in considerable quantity, but was commencing in the 10-day culture, where the glucose had become reduced to 0.01 per cent. Even here there was only a trace of soluble nitrogen present. This is in harmony with our entire experience, in which we have rarely if ever observed more than the low order of 0 to 2 per cent of the total cell nitrogen as soluble nitrogen in media surrounding cells and still containing sugar, or maintained for a week at low temperatures in the absence of sugar. In the Warburg vessels, as in the Erlen-

meyer flasks experiments, the pH tends to increase as considerable amounts of NH_3-N form. This is true whether the CO_2 produced simultaneously is absorbed into alkali or not.

Extent of NH_3-formation. Tables 9 to 12 and figure 1 present a large number of determinations showing that a remarkably high proportion of NH_3-N is obtainable from Azotobacter cells upon spontaneous decomposition. In the

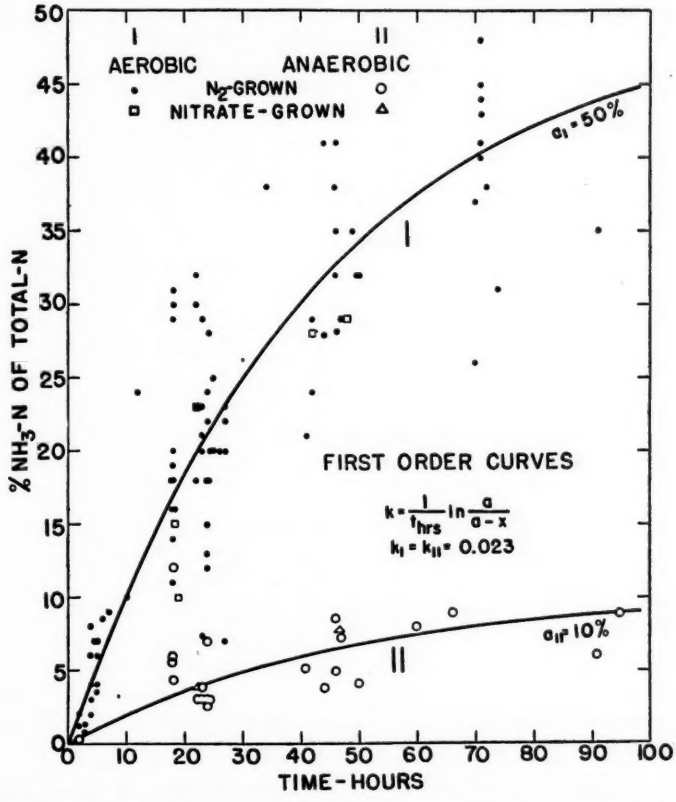

FIG. 1. TIME COURSE OF AEROBIC AND ANAEROBIC FORMATION OF NH_3 FROM CELLS GROWN IN FREE NITROGEN AND NITRATE NITROGEN

Values taken from tables 9, 10, and 11, based on experiments all conducted in the Warburg apparatus at approximately optimum conditions, namely, with substrate-free medium at pH 8.0 ±0.5 and at 31°C.

absence of organic substrate, and under approximately optimum conditions of pH and temperature, a maximum of about 50 per cent of the total cell nitrogen may be transformed to NH_3-N aerobically; and 10 per cent anaerobically, or an extent of 20 per cent as great. About 80 per cent of the transformable nitrogen thus requires external oxidation to form NH_3-N.

TABLE 9

Influence of age of culture, washing, and centrifuging on cell-nitrogen elution and NH_3-formation at different pressures of oxygen

	AGE (DAYS)					
	1	2	3	4	10	
Stock culture*						
pH	6.7	7.0	6.8	6.8	7.0	
NH_3-N, mgm./l.	0	0	0	0	1	
Glucose, per cent	>0.2	>0.2	>0.2	>0.2	0.01	
Filtered centrifugate*						
pH	6.6	6.9	6.8	6.8	6.9	
NH_3-N, mgm./l.	0	0	0	0	1	
Glucose, per cent	>0.2	>0.2	>0.2	>0.2	0.01	
Total-N, mgm./l.	0	0	0	0	2.5	
					(Medium)	Distilled H_2O*
Filtered first and second washings*						
pH	7.1	7.0	7.0	7.0	7.0	7.2
NH_3-N	0	0	0	0	0	0
Glucose, per cent	0	0	0	0	0	0
Total-N, mgm./l.	0	0	0	0	0	0
Stock suspension* (71 hours in Warburg vessels)						
Final pH						
21 per cent O_2 in N_2	8.8	8.6	8.8	8.6	8.5	8.6
21 per cent O_2 in H_2	8.8	8.8	8.8	8.6	8.5	8.6
100 per cent O_2	8.8	8.8	8.7	8.6	.....	8.5
Average	8.8	8.7	8.8	8.6	8.5	8.6
NH_3-N, per cent of total-N						
21 per cent O_2 in N_2	43	39	42	45	50	44
21 per cent O_2 in H_2	43	46	42	45	44	44
100 per cent O_2	43	39	35	45	50	44
Average	43	41	40	45	48	44
Total O_2 consumption (cmm./0.1 mgm. cell-N)						
21 per cent O_2 in N_2	285	272	293	267	311	268
21 per cent O_2 in H_2	291	331	336	308	387	351
100 per cent O_2	300	275	351	287	335	316
Average	292	293	327	287	340	312
cc. O_2 per mgm. NH_3-N (average)	6.8	7.1	8.2	6.4	7.1	7.1

* Stock (glucose) cultures grown for progressively shorter periods of time, each older culture serving to inoculate the next younger. After analysis (without filtration) for pH, NH_3-N, and glucose, 80 cc. of each culture (160 cc. of 10-day culture), with 50–300 mgm. cell-N per liter, was centrifuged, under refrigeration below 30°C. The liquid centrifugate was retained for analysis, and the solid residue washed (30–60 minutes) twice by taking up in 80 cc. inorganic medium (diluted fivefold with distilled water), or in distilled H_2O (0.2 gm./l.

TABLE 9—*Concluded*

phosphate buffer), and centrifuging, this cycle being repeated once again. The clear liquid residues of the washings were combined (for filtration and analysis), and the remaining solid residue was taken up in diluted inorganic medium as a concentrated stock suspension ready for study in the Warburg apparatus. For this purpose they were all diluted to known, approximately the same, concentrations of about 0.06 mgm. cell (micro-Kjeldahl) nitrogen per cubic centimeter. They were diluted with inorganic medium containing 5 gm./l. phosphate buffer at pH 8.0. Each culture was studied in the Warburg apparatus (temperature, 31°C.) in three different gas phases (as indicated), and final NH_3-N and total oxygen consumption were measured. The duration of 71 hours permitted about 90 per cent of complete NH_3-formation and oxygen consumption. Before being analyzed, the centrifugate and combined washings were filtered through a thin, well-washed layer of celite to remove the last traces of cells. The celite as used had little or no effect on the chemical compositions of the fluids. pH was determined colorimetrically, glucose and NH-N by Nesslerization, and total-N by macro-Kjeldahl (sensitive here to 1.5 mgm./l.).

As indicated in table 9, after prolonged incubation, about 7 cc. O_2 are consumed per milligram NH_3-N obtained, or 1 cc. O_2 per 1 mgm. cell dry matter (14 per cent N) initially present. This corresponds to oxidation of protein matter, which requires per unit weight of dry material somewhat less O_2 than, say, sugar, where 1 cc. oxidizes 1.25 mgm. dry matter. Aerobic NH_3-formation is clearly related to oxidation of cell material. A confirmation of this is obtained from the fact that, as will be detailed elsewhere (14), the respiratory quotient (CO_2 produced/O_2 consumed) approaches a value of 0.8 to 0.9. In the earlier periods of incubation the R.Q. is at first 10 to 20 per cent greater than unity but gradually falls to the value indicated. No carbon dioxide is formed anaerobically even under prolonged incubation (91 hours).

Dry weight determinations show that the formation of NH_3-N is accompanied by a parallel loss in cell dry matter, and that at complete decomposition, with 50 per cent NH_3-N of total cell-N, the dry matter is reduced to about 50 per cent (table 16).

It is an interesting fact, although not necessarily an unexpected one, that the microscopic appearance of the cells viewed unstained under the microscope is little changed even after loss of 50 per cent of the cell nitrogen as NH_3-N.

Influence of gas phase. Hydrogen and nitrogen gases are *completely inert* with respect to rate and amount of NH_3-formation and of respiration. The influence of oxygen pressure on cell oxidation is uniform over the range of about 0.01 to 1 atmosphere. This is in marked contrast to the striking oxygen pressure function obtained in respiration with glucose (etc.) as substrate, where Q_{O_2} may be some four hundred times as large, 1000 to 5000, and the optimum occurs at 0.15 atmosphere O_2, and 30 per cent of optimum rate at, say, 0.01 and 1 atmosphere (5).

The time course of NH_3-formation. Figure 1 summarizes all the data of tables 9 and 10 on the rate of formation of NH_3-N from Azotobacter cells grown in free nitrogen. It is seen that a first order velocity curve with a constant of 0.023 (time in hours) fits both the aerobic and anaerobic course,

TABLE 10

Aerobic and anaerobic production of NH_3 from cells of Azotobacter grown in free nitrogen

STOCK SUSPENSION			N IN DRY MATTER	BILLIONS OF CELLS PER		Q_{O_2}†	EXPERIMENT NO.	STORAGE	DURATION	PER CENT NH_2N OF TOTAL-N		ANAEROBIC / AEROBIC
No.	Species*	Age		Mgm. dry matter	Mgm. N					Aerobic	Anaerobic	
		days	*per cent*					*days*‡	*hours*			*per cent*
III	chr.-v.	15	—	—	—	—	2	0	27	7		
IV	chr.	12	—	—	4.8	9	3	0	3, 23	1.3, 7.3	—, 3.0 (N_2 or H_2)	42
V	chr.	¾	15	1.32	7.5	12	4	0	3, 22, 27, 46	0.8, 18, 22, 28		
VI	chr.	¾	8.7	0.65	7.5	16	5	0	2, 22, 70	1.2, 23, 37		
VIII	chr.	19	14.3	0.67	4.7	6	8	1	18, 24, 46	18, 24, 32		
						7	9–10	6	2, 5, 27, 50, 95	2, 3.5, 23, 32	—, —, —, 4.1, 8.7 (N_2)	13
						7	11	8	18, 46	18, 38		
						—	12	10	18, 46	19, 41		
IX	chr.	5	13.3	0.86	6.5	18	13	0	18, 42	8, 24		
XIII	chr.	13	14.0	0.32	2.3	8	14	0	18	16		
						10	16	2	18	17		
XIV	chr.-v.	9	—	—	4.5	10	17	1	18	20		
						6	18	2	18	30		
						7	19	4	18, 66	29, —	6, 9 (N_2)	7, —
						8	20	7	4, 18	6, 31	—, 12 (N_2)	39
XV	chr.-v.	14	10.3	1.05	9.8	—	22	0	18	11	4.3	39
						12	23	4	5, 23	7, 20		
						10	24	9	5, 24	7, 18		
						—	25	10	24	22		
XVI	v.	12	19.8	0.76	3.8	16	28	0	24, 44, 91	13, 28, 35	2.5, 3.8, 6.0 (N_2 or H_2)	19, 15, 17
						16	29–30	5	24, 46	12, —	3, 5 (N_2)	16, —
						14	31	20	4, 7, 12, 32, 34	3, 9, 24, 30, 38		
						13	32–33	15	4, 23	2, 23	—, 3	13
						13	34	21	10	10		

						13	34	21	10	10		
XXIII	v.	21	14.1	0.87	6.4	6	37	5	4, 24	4, 32		
						—	38	7	22, 44	23, 41	3.9, 5.1 (N_2)	17, 13
						5	44	21	2, 25	—, 25	0.3, — (N_2)	
						7	46	27	23, 49, 60	29, 35, —	—, —, 8	
						—	47	28	18, 42	14, 29	5.7, —	41, —
						—	48	32	18, 41, 70	16, 21, 26		
XXIV	v.	17	16.7	0.34	2.3	14	49	1	24, 72	16, 38	7, —	44
						14	50	7	24, 47	21, 29	—, 7.3	—, 26
						18	55	22	27, 74	20, 31		
						14	56	23	25, 50	20, 32		
						18	57	25	24	28		
						16	58	31	26, 46	20, 35	—, 8.7	—, 44
						21	61	48	4	8		
XXIX	v.	26	13.8	0.66	4.8	5	64–65	4	5, 24	4, 18	—, 3.0	—, 16
						—	71	7	5, 25	6‖, 20		
						—	72	12	6	8¶		
XXX	v.	1	—	—	—	(10)	73	1	71	43**		
XXXI	v.	2	—	—	—	(6)	73	1	71	41**		
XXXII	v.	3	—	—	—	(5)	73	1	71	40**		
XXXIII	v.	4	—	—	—	(7)	73	1	71	45**		
XXXIV	v.	10	—	—	—	(7)	73	1	71	48**		
XXXV	v.	10	—	—	—	(6)	73	1	71	44**		
Average.....			13.8	0.75	5.4	10.7						26

Temperature 31°C.; pH 8.0 ±0.5; stock suspensions obtained (as in table 9) by centrifuging air-grown stock cultures and washing twice with (substrate-free) inorganic medium diluted five fold.

* chr. = *chroococcum*; v. = *vinelandii*; chr.-v. = mixture of cultures of the two species made after growth period and just prior to centrifugation.

† Q_{O_2} = cmm. O_2 consumed per mgm. dry matter per hour at start of incubation for NH_3-formation (usually based on readings for first hour).

‡ Time elapsed from preparation of stock suspension until start of incubation for NH_3 formation, the suspension being maintained meanwhile in the ice-chest at about 5°C.

§ Oxygen-free gas employed, N_2, or N_2 or H_2, as indicated in parentheses following value.

‖ Same value obtained in air, and also in 21 per cent O_2 in H_2.

¶ Same values obtained in O_2, air, 21 per cent O_2 in H_2, 5 per cent O_2 in N_2, 5 per cent O_2 in H_2, at three different cell concentrations (table 12).

** Same values obtained in air, 21 per cent O_2 in H_2, and 100 per cent O_2 (table 9); cell dry matter not determined for XXX–XXXV, and Q_{O_2} estimated on basis of 14 per cent N.

TABLE 11

Aerobic and anaerobic production of NH_3 from cells of Azotobacter grown in fixed nitrogen

STOCK SUSPENSION			N IN DRY MATTER	Q_{O_2}†	EXPERIMENT NO.	STORAGE	DURATION			PER CENT NH_3-N OF TOTAL-N									ANAEROBIC / AEROBIC		
										Aerobic (in air)§						Anaerobic (in N_2)					
No.	Species*	N-Source for growth								(5	24	48	72	114	162)						
			per cent			*days*‡	*hours*												*per cent*		
X‖	chr.	urea	15.2	18	13	0	18	42			4	23									
XI‖	chr.	ammonia	15.9	—	13	0	18	42			1	18									
XII‖	chr.	nitrate	15.2	18	13	0	18	42			15	28									
					15	7	22				23					4			17		
							47									8					
XVII	v.	glutamate	13.4	15	36	0	19	48			1	9									
					36a	5	19				2										
XVIII	v.	asparagine	14.0	15	36	0	19	48			7	23									
XIX	v.	nitrate	13.6	16	36	0	19	48			10	29									
					36a	5	19				16										
XX	v.	adenine	15.0	17	36	0	19	48			7	22									
XXI	v.	creatine	16.4	13	36	0	19	48			5	22									
					36a	5	19				4										
XXII¶	v.	mixture¶	14.2	18	39	13	5			3											
				15	40	14	4			2											
				—	41	15	18				12										
				14	42	16	20	67			12					3.6	7.1		30		
				21	43	21	73						45			9			20		
XXV	v.	glutamate	13.1	13	59	0	19	45	68		3.2	9.5	18								
				13	60	5	19	43	71		5.0	11	20								
							114	162						28	36						
					60a	8	27	49	96							1.1	2.1	2.9	(—	20	13)
					61	13	5			1.8											

					61	13	5			1.8										
XXVI	v.	creatine	16.7	8	59	0	19	45	68		3.6	10	20							
XXVII	v.	alanine	12.7	14	59	0	19	45	68		2.8	8	13							
								114	162					22	26					
				16	60	5	19	43	71		3.6	8.5	14							
					60a	8	27	49	96							0.9	1.7	2.5	(—	20	15)
					61	13	5			1										
XXVIII	v.	peptone	12.0	18	59	0	19	45	68		0.4	6	19							
				17	60	5	19	43	71		0.4	5.6	22							
								114	162					34	34					
					60a	8	27	49	96							1	2	—	(—	33	—)
					61	13	5			0										
Average.....			14.4	15.4						1.5	6.6 4.4**	15 14**	21	28	32				21 21**		

Temperature, 31°C.; pH 8.0 ±0.5; all cultures used in preparing stock suspensions, 5 days old; cultures for X, XI, and XII grown simultaneously, also for XVII–XXII, and for XXV–XXVIII; stock suspensions obtained (as in table 9) by centrifuging stock cultures and washing twice with (substrate-free) inorganic medium diluted fivefold; Q_{O_2} determinations averages of three essentially agreeing triplicates, run usually at three different cell concentrations.

* chr. = *chroococcum*; v. = *vinelandii*.

† cmm. O_2 consumed per mgm. dry matter per hour at start of incubation for NH_3 formation.

‡ Time elapsed from preparation of stock suspension until start of incubation for NH_3 formation, the suspension being maintained meanwhile in the ice-chest at about 5°C.

§ Columns arranged in order of approximate durations of 5, 18–28, 43–50, 67–71 (and 96), 114, and 162 hours.

‖ Cultures for these suspensions grown simultaneously, and closely comparable with, culture for suspension IX, table 10; aerated with a stream of air (compressed); all other cultures of this table grown in O_2, or a bomb mixture of 50 per cent O_2 in N_2.

¶ XVIII, XIX, and XX combined after use in experiments 36 and 36a.

** Nitrate-grown cultures excluded.

with only the end points, 50 per cent and 10 per cent NH_3-N, differing. To what extent, if any, aerobic deamination is limited by the anaerobic step, or to what extent it may be considered that the curves indicate a common rate of enzyme decomposition, remains to be determined.

The velocity constant of 0.023 corresponds to an initial rate of decomposition of 1 per cent of the total cell nitrogen per hour, or 2 per cent of the cell nitrogen eventually decomposed. Whereas this rate is only moderate compared with many biochemical and chemical reactions, from the agronomic standpoint it is very rapid and means that a large amount of the nitrogen fixed by Azotobacter can be liberated into the soil, almost without significant time limitation, once optimum conditions occur. Thus 50 per cent of the nitrogen available

TABLE 12

Influence of different gas phases on NH_3-formation

GAS PHASE	FINAL pH	O_2 CONSUMED IN 5 HOURS PER 10 MGM. CELL DRY MATTER	Q_{O_2} CMM. O_2/MGM./HR.	NH_3-N 5 HOURS
		cmm.		*per cent*
100 per cent O_2	8.1	278	5.6	8.0
21 per cent O_2 in N_2	8.1	272	5.4	8.0
21 per cent O_2 in H_2	8.1	270	5.4	8.0
5 per cent O_2 in N_2	8.1	254	5.1	8.0
5 per cent O_2 in H_2	8.1	291	5.8	8.0
0.75 per cent O_2 in N_2	7.9	176	3.5	6.0

Stock suspension XXIX, temperature 35°C., initial pH 8.0, NH_3-N estimated colorimetrically. Determinations are averages of essentially agreeing triplicates at cell concentrations of 8, 4, and 2 billion per cc. in 1, 1.5, and 2 cc. volumes of culture, respectively, or 12.2, 9.15, and 6.1 mgm. cell dry matter per Warburg vessel (1.69, 1.26, and 0.84 mgm cell-N per vessel). There is no influence on Q_{O_2} or NH_3-N of either H_2 or N_2, or of varying O_2 pressures between 100 and 5 per cent, but at 0.75 per cent O_2, because of diffusion limitation or otherwise, the NH_3-N is reduced 25 per cent and similarly the respiration about 35 per cent.

for release as NH_3-N (25 per cent of the total cell-N) would be liberated aerobically in 1 day, and 80 per cent within 3 days.

A remarkable feature of the time course is the absence of lag or induction. When suitable optimum conditions are provided the process of decomposition sets in immediately in a manner that can be characterized by usual and simple physicochemical treatment. This point is highly significant in connection with the nature and general certainty of the process.

The rather large spread of points in figure 1 is due partly to small but definite deviations from strictly optimum conditions (pH variation of 1 unit, etc.), and also to aliquoting and to accumulative errors in NH_3-N and total-N determinations. The points represent chiefly the controls in some 70 different experiments, using 20 different stock suspensions, and carried out over a period of a year; from this point of view the spread is in reality remarkably small, rather than large as might appear at first glance. It will be seen, for instance, that although

the average Q_{O_2} is 15.4 (table 10), there is a variation, among the stock suspensions, of from 5 to 20; and similarly the average "billions of cells per milligram of dry matter," 0.75, varies from 0.34 to 1.32; and the average "billions of cells per milligram of cell-N," 5.4, varies from 2.3 to 9.8. This variation in the nature of the cells of different stock suspensions is large or small, depending upon one's point of view. Although the nitrogen content of the dry matter of the cells was usually close to 14 per cent, a variation from 8.7 to 19 per cent was observed. In this latter connection, as is well known, cultures grown on agar usually have much lower nitrogen contents of 2 to 5 per cent (11), due merely to incidentally accompanying carbohydrate or gum. With any one stock suspension duplication of results was of course usually very satisfactory (tables 10, 11). Also, very carefully conducted individual experiments gave first order (logarithmic) curves in the same manner as the grand average of figure 1.

The age of cultures maintained in organic substrate, varied up to 30 days, or the period of storage of suspensions maintained at low temperature and without organic substrate, varied up to 60 days, had practically no influence on the rate or extent of NH_3-formation or cell oxidation. This provides strong incidental support for the straightforward physicochemical nature of the process indicated in figure 1 and shows that Azotobacter cells are at all times ready to yield NH_3 under proper conditions. Still further support for this view may be drawn from the fact that cell concentration was without consistent influence upon either the rate or the extent of ammonia formation per unit amount of cells. In this connection the concentration was varied from 0.1 to 20 billions per cubic centimeter. There is a direct relation between yield of NH_3-N and cell mass or cell-N; *the cells thus act as sources of NH_3-N and not essentially as catalysts for direct production of NH_3-N from some other source, such as N_2 and glucose.*

In the same way it has been impossible to detect a statistically significant difference in regard to NH_3-formation and related phenomena between the strains of *A. chroococcum* and *A. vinelandii* used. The two species types (11, 17, 26) could, nevertheless, be clearly distinguished upon other grounds; they maintained consistently, even after several years' cultivation on laboratory media, the different pigment formations necessary for their differentiation *A. chroococcum*, black pigmentation, *A. vinelandii*, green or pink coloration (17). Green (11) found *A. vinelandii* to contain a considerably lower percentage carbohydrate and higher percentage of nitrogen than *A. chroococcum*, but fractionation of the nitrogen showed no essential differentiation.

As other incidental factors, it may be mentioned that, in general, shaking the incubating suspensions, as in the Warburg apparatus, was without influence so long as diffusion limitation of oxygen was avoided. Except for any minor changes in pH involved, the rate of NH_3-formation was independent of the presence of (CO_2-absorbing) alkali in the wells of the Warburg vessels and also of H_2SO_4 placed in the side-arms. The H_2SO_4 would not usually absorb the NH_3 as fast as it was formed in the culture in the main part of the vessel, and so could not be used with great advantage in measuring the NH_3-N formed.

Cells grown in fixed nitrogen. Table 11 shows that, in principle, and for the

most part in detail also, cells grown in widely varying forms of fixed nitrogen yield NH_3-N in the same manner as cells grown in free nitrogen. Aerobically and anaerobically, limits of 50 and 10 per cent of total cell-N are obtained as NH_3-N, after sufficient incubation. The nitrogen content in the dry matter averages 14.4 per cent (instead of 13.8 in table 10). Q_{O_2} averages 15 instead of 11 (probably not significantly different, statistically).

The only definite distinction noticeable is in regard to a slower rate of NH_3-formation with *certain* of the nitrogen compounds. Nitrate-N gave cells indistinguishable from those grown in free N_2-N (fig. 1 and table 2). However, it would appear that compared to the nitrogen sources, N_2 and nitrate, the other nitrogen compounds gave suspensions which yielded NH_3N at an initial rate, for the first 10 to 20 hours, about one third as great, as a grand average. As a matter of fact, however, the entire series may be arranged in order of rate of formation of NH_3-N, *inversely* as the following approximate order of the rates of growth of the particular original stock cultures from which the stock suspensions were derived, thus: group 1, peptone, ammonia, urea, and glutamate; group 2, alanine, and creatine; group 3, asparagine and adenine; group 4, nitrate and N_2. This is true both for aerobic and for anaerobic decomposition. Many other phenomena of Azotobacter cell physiology show this inverse relationship with rate of growth; in this connection, for example, may be cited without further discussion the stimulation by soil humic acid, the effect of temperature upon stimulation by soil humic acid, the toxic effect of high oxygen pressures, and the rate of respiration in the presence of glucose.

The following values of "per cent NH_3-N of total-N in first 24 hours" will give some idea of the approximate relative initial rates under aerobic conditions: groups 1, 2, 3, 4: 0.5 to 2, 3 to 5, 6 to 10, 15 to 25 per cent, respectively. With groups 1 and 2 (and to some extent 3) there is a definite induction period, and the rate-time curve is concave upward at first, instead of concave downward (logarithmic) as in figure 1 for group 4. After the lag period the specific velocity constants approach but never exceed the value for group 4 (of about 0.02). Upon repetition of experiments with given nitrogen sources, the arrangement of the four groups would appear to be reasonably consistently maintained; but incidental growth factors not controlled entirely (rate of aeration, duration of growth, size of inoculum, etc.) no doubt also played a minor rôle in the particular results reported in table 11.

It is possible to interpret the inverse relation discovered, upon the *ad hoc* view that the faster rate of growth indicated a more ready utilization of a given nitrogen source and hence a greater opportunity to build the nitrogen into somewhat more complex, less easily broken down compounds. Since, however, the final amounts of NH_3-N formed are independent of nitrogen source, it is much more probable that the enzyme systems concerned in breakdown are not equally developed, a physiological phenomenon now recognized to be of very common occurrence. This latter view is supported by the fact that, in general, when rates of NH_3-formation 30 per cent suboptimal or less are involved, as, for instance, a function of pH or temperature, the time curves are likewise of the concave upward type involving more or less induction or lag.

In any case, it is evident that, as might be expected, although there are significant differences detectable between cultures grown in different nitrogen sources, they are of an order not so much greater than differences between cultures grown at different times on the same nitrogen compound. For all practical purposes, then, NH_3-formation by cells grown in free and in various forms of fixed nitrogen must be regarded as essentially the same process, as far as observed. In view of the large NH_3-formation involved this result obviously has a very pertinent bearing on the question of the origin of any NH_3-N obtained from cultures grown in free N_2 and strongly suggests that all of the NH_3-N so obtained is derived immediately from cell nitrogen and only remotely, from a mechanistic point of view, from N_2.

The inhibition of NH_3-formation by organic substrate

In this section will be shown in a very decisive manner, in several different ways, the striking inhibition of NH_3-formation by oxidizable organic substrate. In all cases it will be evident that the mechanism of inhibition operates through inhibition of the oxidation of the cell material itself, which, as indicated earlier, is intimately tied up with aerobic NH_3-formation.

Concentration function. It was found with the Warburg technique that, just as in the Erlenmeyer flask technique, no NH_3 formed until the oxidizable substrate had decreased to a certain very low value which was ascertained not only chemically but also by calculations based on the observed total oxygen consumption. The same limiting, low value of about 0.01 to 0.03 per cent substrate was found. It was also found that this value could be interpreted in terms of respiration rate. Thus, in general, inhibition of NH_3-formation became easily detectable just as soon as the organic substrate increased the rate of oxygen consumption by 25 per cent or more over that given by the cell material itself. The functions involved are clearly demonstrated in figure 2, where reciprocal relations between NH_3-formation and respiration rate *in the presence of substrate* are shown to occur over the same concentration range, 0.01 to 0.03 per cent, the same range, in fact, that was observed in the Erlenmeyer flask technique. Glucose and succinate affected the two processes identically. With organic substrates which are oxidized less readily than these, the concentration range would be shifted higher for both processes. In other words, the actual concentration range providing inhibition of NH_3-formation is significant insofar as it is the concentration range which significantly increases the cell respiration. Evidence will be provided elsewhere (14), in connection with studies on the respiratory quotient, that the organic substrate is competitively oxidized in preference to cell material, and that the oxidation of the latter (along with NH_3-formation) is partially or completely suppressed.

Substrate groupings. Table 13 shows, for many different organic substrates, the same sort of inhibition as that obtained in figure 2 with succinate and glucose. Here the quantitative relations are not demonstrated with quite the precision of figure 2, since the respiration rates are given for the first and eighteenth hours only. It will be seen (in XIII-14, XIII-16, XIV-17, or IV-3) that wherever the rate of oxygen consumption is increased at either of these periods, NH_3-formation is either partially or completely inhibited. In some cases, at low concentrations, respiration increase is observed at the first hour

but not at the eighteenth hour, because the organic substrate had by this time been completely consumed. In other cases marked respiration increase was noted at the eighteenth hour but none at the first hour because of a lag of several hours involved with some organic substrates before they are utilized. These lag periods have been studied and portrayed in detail by Lineweaver (25). Respiration rate values are given in table 13 for two standardized, significant periods of time only; namely, when NH_3-formation was just commencing, and

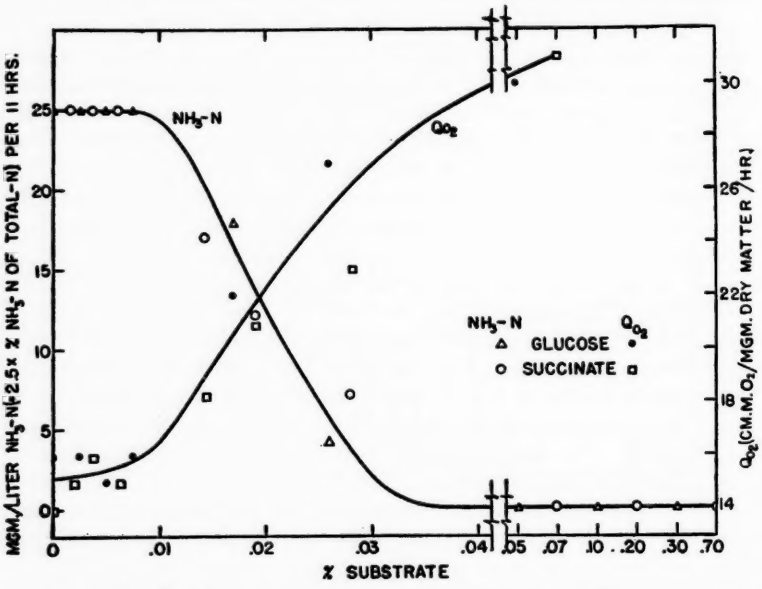

FIG. 2. INFLUENCE OF CONCENTRATION OF SUBSTRATE UPON RATE OF NH_3-FORMATION AND RATE OF RESPIRATION

1.5 cc. per Warburg vessel of stock suspension XVI (table 10) diluted to contain 250 mgm./l. cell nitrogen, and maintained at pH 8 and 31°C. Duration of experiment, 11 hours; Q_{O_2} given for eleventh hour. Substrate concentrations corrected for slight decreases due to substrate consumption (above 0.01 per cent, 15 per cent correction at most, on a time average). Succinate refers here to succinate ion (M.W. 116), not to sodium succinate (M.W. 162) as otherwise usual.

when 40 to 60 per cent completed, but in these and most of our other experiments measurements were taken periodically throughout the whole time course.

It may be said in regard to oxidation of cell material in the absence of substrate that, in general, the rate of oxygen consumption or, better, the total oxygen consumption, followed the same rough time course as NH_3-formation shown in figure 1. For instance, in one particular experiment, out of over

TABLE 13

Influence of added organic substrates on respiration and on aerobic and anaerobic NH_3-formation in stock suspensions

GAS	STOCK SUSPENSION No. and experiment	SUBSTRATE	CONCENTRATION per cent	pH 18th hour	Q_{O_2} 1st hour	Q_{O_2} 18th hour	NH_3-N, 18TH HOUR mgm./l.	NH_3-N, 18TH HOUR Per cent of total-N	PER CENT CHANGE, 18TH HOUR Q_{O_2} (increase)*	PER CENT CHANGE, 18TH HOUR NH_3-N (inhib.)
Air	XIII-14	.....	.....	8.6	8	8	65	15	.....	...
		Sucrose†	0.01	8.6	8	8	65	15	0	0
			1.0	7.6	10	62	0	0	620 (125)	100
		l-arabinose‡	0.001	8.7	8	8	65	15	0	0
			0.1	8.7	8	8	65	15	0	0
			1.0	8.4	8	8	65	15	0	0
		Maltose†	0.01	8.7	8	8	65	15	0	0
			1.0	7.0	12	86	0	0	720 (150)	100
Air	XIII-16	.....	.....	8.7	12	10	75	17	.....	...
		Levulose†	0.05	8.6	35	10	75	17	.... (280)	0
			0.2	8.0	41	10	4	0.9	.... (350)	85
			1.0	7.7	62	53	0	0	530 (520)	100
		Na-succinate†	0.05	9.1	20	10	65	15	.... (160)	8
			0.2	9.3	26	24	18	4	240 (240)	75
			1.0	9.2	39	77	0	0	770 (330)	100
		Na-acetate†	0.05	8.9	11	14	75	17	140	0
			0.2	9.2	12	18	65	15	180	8
			1.0	9.2	20	105	0	0	1050 (160)	100
Air	XIV-17	.....	.....	8.5	6	6	90	20	.....	...
		Na-glycerophosphate‡	0.05	8.6	6	6	90	20	0	0
			0.2	8.6	6	6	90	20	0	0
			1.0	8.0	8	6	75	17	0 (125)	15
		Na-benzoate†	0.05	8.7	6	6	75	17	0	15
			0.2	8.6	6	9	35	8	150	40
			1.0	8.1	6	46	0	0	760	100
Air	XXIII-37	.....	.....	8.3	..	17	104	32	.....	...
		Na-maleate§	3.2	8.2	..	0.3	23	22	—98	78
		Na-oxalate§	1.34	8.2	..	7	65	21	—62	38
			2.7	8.8	..	1	18	17	—95	83
		Na-glycollate§	1.0	8.3	..	3	45	43	—83	57
			10.0	8.2	..	0	13	12	—100	88
		Na-formate§	0.7	9.0	..	4	35	34	—76	66

Q_{O_2} as cmm. O_2/mgm. cell dry matter/hour, NH_3-N determined by Nesslerization, pH values colorimetric. Initial pH 8.4. Temperature 31°C. In experiments 7 and 3, the duration was not 18 hours as indicated, but 72 and 25 hours respectively.

* Values in parenthesis indicate per cent increase in Q_{O_2}, if any, at first hour.

† Substrates oxidized and inhibiting NH_3-formation.

‡ Substrates not oxidized and not inhibiting NH_3-formation.

§ Substrates not oxidized and inhibiting NH_3-formation.

TABLE 13—*Concluded*

GAS	STOCK SUSPENSION No. and experiment	SUBSTRATE	CONCENTRATION per cent	pH 18th hour	Q_{O_2} 1st hour	Q_{O_2} 18th hour	NH_3-N, 18TH HOUR mgm./l.	NH_3-N, 18TH HOUR Per cent of total-N	PER CENT CHANGE, 18TH HOUR Q_{O_2} (increase)*	PER CENT CHANGE, 18TH HOUR NH_3-N (inhib.)
Air‖	IV-3	.....	.....	7.8	9	9	100	7.3	.....	...
		Glucose†	1.0	7.5	32	105	0	0	1150 (350)	100
N_2 or (H_2)	IV-3	.....	.....	7.4	..	.....	40	3.0	.....	...
		Na-succinate	1.0	7.4	..	.....	40	3.0	.....	0
		Glucose	1.0	7.4	..	.....	40	3.0	.....	0
N_2	VIII-7	.....	.....	7.2	..	.....	35	3.5	.....	...
		Na-formate	0.5	7.4	..	.....	35	3.5	.....	0
N_2	XVI-33	.....	.....	7.8	..	.....	30	3.0	.....	...
		Glucose	1.0	7.8	..	.....	30	3.0	.....	0

‖ These two experiments (aerobic and anaerobic) performed simultaneously.

five hundred, the rates of oxygen consumption at 1, 44, and 91 hours were 80, 20, and 8 cmm. O_2/vessel/hour, respectively (Stock Suspension XVI). The same correspondence between courses of respiration and of NH_3-formation was also observed with fixed nitrogen-grown cultures, even where induction and several fold longer incubations were involved. In the cases of a very few cultures the rate was occasionally observed to increase for the first 24 hours, as though some less readily available stored cell substance, such as gum, was being broken down into a more readily available material.

Table 13 presents some very interesting qualitative groupings of organic substrates. There are substrates, like glucose or succinate, which are oxidized and inhibit NH_3-formation in the manner already described. There are also substrates (arabinose, glycerophosphate) which are not appreciably oxidized, and which, in accordance with our now developed, general understanding and theory should not, and do not, inhibit NH_3-formation. A third group (maleate, oxalate, glycollate, and formate) are not appreciably oxidized, but independently, at comparatively very high concentrations, inhibit both NH_3-formation and oxidation of cell material. Finally, under the imposed anaerobic conditions, where oxidation of both substrate and cell material is prevented, substrate inhibition is in no instance observed. (This is particularly interesting in the case of those substrates shown which, under aerobic conditions, are oxidized and inhibit NH_3-formation.) These four qualitative groupings show independently, in a very striking and effective manner, the important parallelism, a relation of cause and effect, between oxidation of cell substance and formation of NH_3-N.

The fact that oxidation of organic substrate completely inhibits NH_3-formation aerobically, shows that anaerobic NH_3-formation has to be understood as more than simple hydroly-

sis, otherwise the underlying process should occur under aerobic conditions also and one should thus obtain a yield of some 20 per cent of the transformable nitrogen [10 per cent of the cell-N (fig. 1)]. Certain oxidation-reduction relations may be involved, as is now known to be the case with some deaminases (e.g., urease, arginase); or some oxidation intermediate may inhibit the process analogously to O_2 in the Pasteur-Meyerhof reaction (aerobic inhibition of fermentation).

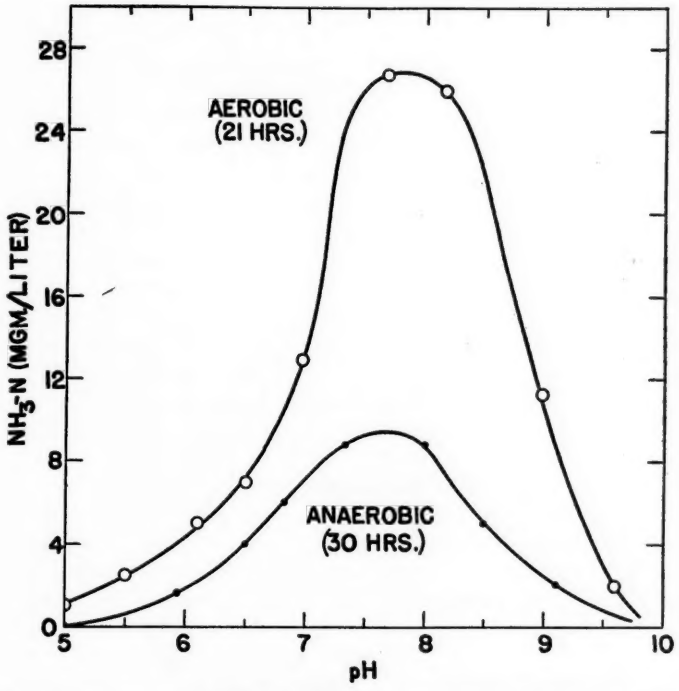

FIG. 3. INFLUENCE OF pH UPON THE AEROBIC AND ANAEROBIC FORMATION OF NH_3

1.5 cc. per Warburg vessel of stock suspension XVI (table 10) diluted with inorganic (substrate-free) medium, containing 10 gm. phosphate mixture, to 260 mgm./l. cell nitrogen, and maintained at 31°C. Anaerobic cultures maintained in O_2-free N_2. Initial and final pH values essentially identical.

Secondary and incidental factors in NH_3-formation

pH. Figure 3 presents the pH functions obtained for aerobic and anaerobic NH_3-formation when all other factors are held essentially at optimum and the organic substrate, in particular, is eliminated from the medium. The optimum pH is seen to occur at 7.8 to 8.0, with half maximal values at 6.5 to 7.0 and 8.5 to 9.0; the anaerobic curve is possibly shifted to a slightly more acid range than the aerobic. Of especial interest, too, is the ammonification measurable even below pH 5.0 and above 9.5, particularly upon more prolonged incubation than

that involved in figure 3. For all practical purposes NH_3-formation occurs over a very wide physiological range, and is not limited to a markedly alkaline range, as believed by Winogradsky, or to a mildly acid range, as supposed by Isakova (15). With less controlled technique, as with Erlenmeyer flasks, or

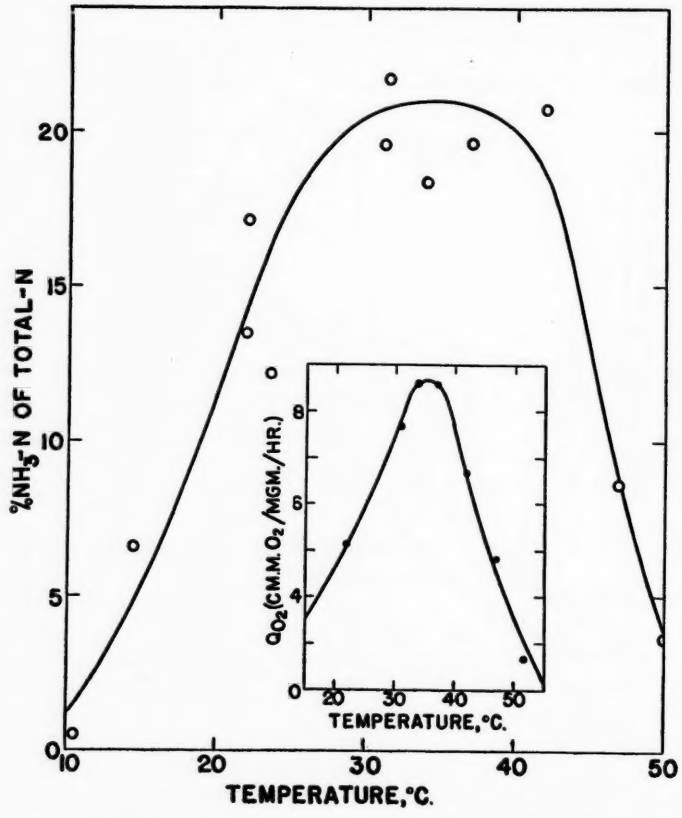

FIG. 4. INFLUENCE OF TEMPERATURE UPON THE RATE OF NH_3-FORMATION AND THE RATE OF RESPIRATION

1 cc. per Warburg vessel of stock suspension XV (table 10) diluted with organic substrate-free medium to contain 1020 mgm./l. cell nitrogen, and maintained at pH 8. Duration of experiment, 24 hours. Q_{O_2}, the rate of respiration in cmm. O_2/mgm. cell dry matter/hour, is based on the average rate for the first 5 hours.

with silica gel media, and with evaporation permitted, we ourselves have been able to observe apparent optima varying from 6.5 to 9.5, due to the operation of comparatively incidental factors. The great majority of deaminases have an optimum activity in the region of pH 8, the same as that found in figure 3.

Although the rate of NH_3-formation is appreciably reduced at pH values greater or less than 7.5 to 8.5, the final amount of NH_3-N produced after somewhat more prolonged incubation is independent of pH, except at the very extreme limits where the rate has been reduced to 10 to 20 per cent or less of the maximum and where induction or irreversible inactivation may occur. Because of this, the rate curves presented in figure 3, particularly the aerobic curve, are possibly a little steeper than curves obtained by measurements not involving a time integration over a number of hours. In any case, the rates remained reasonably constant over the time period required for their measurement, at all except the very limiting pH values.

The curve for rate of oxidation of cell material as a function of pH is similar to that for NH_3-formation, being perhaps a little narrower.

Temperature. Figure 4 shows that, as with pH, the temperature function is very broad, with an optimum between 30° to 40°C., half-maximal values at 20° and 46° to 48°, and limits at 5° and 55°C. or beyond. At low temperatures the same phenomenon of lag (and steepening of curve) is observable as in the case of very low pH values or as with certain slowly decomposing, fixed nitrogen-grown, suspensions described earlier. Thus, in the case of one stock suspension maintained at about 8°C., the following concentrations of NH_3-N were observed to accumulate: 0.3, 0.7, 3.1, 4.6, 9.0, 16 per cent of total-N, after 4, 6, 9, 14, 23, 36 days, respectively. Stock suspensions maintained for a week or more at 0° to 2°C. will show a prolonged lag of many hours upon being incubated at optimum temperature, but after eventual recovery the final amounts of NH_3 formed and cell oxidation are normal.

The curve for respiration rate as a function of temperature (inset, fig. 4) is similar to, although somewhat narrower than, that for NH_3-N. This is true both for rates established without lag and for those at the limits of the function involving induction and again indicates a close relationship between the two processes.

Buffers and salts. At constant, approximately optimum pH (7.5 to 8.5), potassium phosphate mixture gives no indication of toxicity when used up to at least 10 gm./l. Borate ($Na_2B_4O_7 \cdot 10H_2O$), which gives the same pH function for both NH_3-formation and cell oxidation as phosphate, inhibits the rates of both these processes 25 to 75 per cent, at a concentration of only 0.5 to 1 gm./l. The total NH_3-N formed after an extended period of time is not affected, except possibly at 5 to 10 gm./l. of borate. The borate inhibition of rate is not due to phosphate absence because it may also be obtained in a mixture of 5 to 10 gm./l. borate and 1 gm./l. phosphate. Increasing the proportion of phosphate will overcome borate toxicity, however. The rate of NH_3-formation is not greatly affected by the absence of salts in the medium, but proceeds normally in a suspension made up with distilled water and buffered at pH 8 with a small amount of phosphate mixture.

Pasteurization. 15 minutes' pasteurization at 55°C., or 5 minutes' at 75°C. or 95°C., completely inhibits NH_3-formation and cell oxidation for a few hours, but by 10 to 15 hours both processes are restored to almost the initial rate, and the final extent of NH_3-formation and oxidation is unaffected. This is also true even for 30 minutes' treatment at 95°C. The essentially non-vital (strictly enzymic) nature of the processes is thus strongly indicated. A more detailed study of the aspects of recovery would undoubtedly be of considerable interest.

Toluene. A saturated solution of toluene inhibits to the extent of 80 to 90 per cent NH_3-formation and oxidation of Azotobacter cells and also, as will be shown elsewhere (14), to varying degrees the same processes carried out by cells acting upon fixed nitrogen compounds such as aspartate, arginine, guanine, allantoin. In striking contrast, however, the anaerobic formation of NH_3 is entirely unaffected by toluene. Kostytschev and Scheloumova (21) had observed considerable aerobic inhibition of ammonification (and also of fixation). Winogradsky (34) found toluene did not stop NH_3-formation completely. Toluene evidently does not greatly prevent the anaerobic (hydrolytic) process from taking place when O_2 is present, in marked contrast to the action of oxidizable organic substrate, where the inhibition of NH_3-formation may be definitely complete.

Cyanide. NH_3-formation and cell respiration are affected in an identical manner by cyanide, 50 per cent inhibition being obtained at about 10^{-3} *M*. The same is also true with alanine as a substrate, except for greater sensitivity, 50 per cent inhibition occurring at 10^{-4} *M*. According to Lineweaver (25), 50 per cent inhibition of glucose oxidation by cells occurs at a still lower concentration, 5×10^{-6} *M*, whereas anaerobic dehydrogenation of glucose by methylene blue in the presence of Azotobacter cells is not affected at all at even 2×10^{-3} *M*. The effect of cyanide on anaerobic NH_3-formation from Azotobacter cells has been determined to be extremely weak (14). In making the aerobic determinations it was necessary to estimate the respiration in the absence of CO_2-absorbing alkali, otherwise the cyanide would likewise be absorbed completely from the medium in a short time. The experiments with cyanide will be detailed elsewhere (14) in connection with additional experiments with added fixed nitrogen substrates capable of being ammonified.

Nitrate. Kostytschev considered that Azotobacter reduced nitrates directly to NH_3, in the same manner as he held N_2 to be so reduced, and also in the same manner as he and Tzwetkowa (24) and Klein, Eigner, and Müller (16) had observed fungi, and Warburg and Negelein (31) had observed the green alga Chlorella, to do. However, for the same reasons as those involved in the case of N_2, it is impossible to determine from Kostytschev's experiments whether with Azotobacter the NH_3 observed was really derived directly from nitrate or indirectly through formation of normal cell nitrogen.

Using stock suspensions, we have found under aerobic conditions no trace of influence of nitrate on NH_3-formation, either in the presence or absence of glucose or other organic substrate. The nitrate had, in fact, no effect on NH_3-formation from cell-N except at very high concentrations of 1000–2000 mgm./l. nitrate-N or more. NH_3-formation and accompanying cell oxidation are then inhibited. The possibility of direct reduction of nitrate to ammonia by Azotobacter cannot be ruled out, of course, but any previous demonstration is very questionable.

Under strictly anaerobic conditions, not considered by other workers heretofore, the situation is quite different. Here, in the absence of sugar, nitrate reacts as an oxidizing agent with cell material and forms, at concentrations ranging from 5 to 2000 mgm./l. N, increased amounts of ammonia as compared with those obtained in the absence of the nitrate. In the presence of glucose and nitrate, as we have shown (4), growth may take place anaerobically with, incidentally, the same pH function. Under these conditions, the nitrate consumed is partially utilized in growth, so that the net result of adding glucose to anaerobic cell suspensions containing nitrate is usually to decrease the yield of NH_3-N obtained.

The anaerobic production of NH_3 by cells in the presence of nitrate and in

the absence or presence of organic substrate is accompanied, as might be expected, by the formation of carbon dioxide, since the nitrate is providing bound oxygen to oxidize cell material (or substrate) in a manner analogous to O_2 gas under aerobic conditions. It will be recalled that in anaerobic NH_3-formation by cells, in the absence of nitrate, no carbon dioxide is produced.

The relations obtained with nitrate are apparently given, as might also be expected, with nitrite, but to a much less noticeable extent. Under strictly anaerobic conditions nitrite is actually formed from nitrate, as will be considered later (14).

Ammonification of fixed nitrogen substrates

For completeness of perspective, brief mention will be made of results to be detailed elsewhere (14) which show that Azotobacter suspensions are readily able to ammonify, partially or completely, a large number of nitrogenous compounds, including proteins, amino acids, nucleic acids, and simple and substituted amides, in much the same manner, and with much the same rate, as Azotobacter liberates ammonia from its own cell nitrogen. Anaerobic ammonification is somewhat more limited than aerobic, affects only a few amino groups, and is confined chiefly to various amide compounds, which are seldom ammonified completely, particularly in the case of ring compounds. A number of compounds, including certain higher amides, simple amines, substituted ureas, and urethanes, are not ammonified either aerobically or anaerobically and are not even oxidized. In general, oxidizable, non-nitrogenous substrates inhibit aerobic ammonification of nitrogenous substrates in the same variety of ways as they do ammonification of cell nitrogen and, likewise, they have little or no effect under anaerobic condition. The relations with respect to gas phase, pH, and narcotics such as toluene and cyanide are also similar. These and many other unmentioned findings with respect to fixed nitrogen substrates for ammonification all lend much support to conceptions put forward in this paper, and will be shown to provide a close insight into the more strictly chemical aspects of NH_3-formation from cells (14).

DISCUSSION

Quantitative critique

The many and varied results presented here oppose unanimously schemes A and B described in the Introduction, according to which the extracellular ammonia *observed heretofore* is derived wholly or in part by *direct* synthesis from N_2. This statement is made upon the basis of the following new findings demonstrated in this paper: the non-simultaneity of N_2-disappearance and NH_3-appearance; the similar formation of NH_3 by cultures grown in fixed nitrogen and in free nitrogen; the extensive ammonifying capacity of Azotobacter cells acting upon various fixed nitrogen substrates; the close quantitative correlation between NH_3-formation and oxidation of Azotobacter cell material, demonstrated under a great variety of circumstances with respect to amount,

rate, and respiratory quotient; and the quantitative limit for the maximum amount of NH_3 formed in relation to cell material. In this last connection the following analysis of previous investigations used in support of schemes A and B may be made, concluding that given in the Introduction.

The ease and delicacy with which rather small concentrations of NH_3 could be measured and the fact that the total amount of nitrogen fixed was often not measured resulted in a definitely misleading view as to the actual relative amounts formed in relation to the total amount of nitrogen fixed. An examination of all of the published experimental data shows that the yields of NH_3-N obtained actually never amounted to more than a fifth of the reported total nitrogen fixed and were ordinarily one tenth to one twentieth. The significance of this observation, previously overlooked, cannot be overestimated: the quantities of NH_3 obtained were really so small, relatively, as easily to allow of their having been derived *entirely* from fixed

TABLE 14

Experiments of Kostytschev, Ryskaltschouk, and Shvetzova (19) on the formation of NH_3-N and amino-N in Azotobacter cultures grown in free nitrogen

CULTURE NO.	AGE	FINAL NET NITROGEN PER 100 CC.				PER CENT SOLUBLE-N OF TOTAL-N	PER CENT NH_3-N OF TOTAL-N
		Total	Soluble	NH_3-N	Amino-N		
	days	*mgm.*	*mgm.*	*mgm.*	*mgm.*		
8	10	....	3.9	1.6	2.2	....	...
9	10	....	2.2	1.4	...	....	...
10	6	....	3.4	3.1	...	....	...
11	15	16.7	2.7	1.3	1.2	16.2	7.8
12	7	15.7	2.6	0.7	2.0	16.6	4.5
13	9	17.7	2.1	0.3	1.8	19.0	1.7
14	3	6.8	1.1	0.3	0.8	16.2	4.5
15	6	10.5	1.3	0.3	0.9	12.4	2.9
16	12	14.1	1.3	0.2	0.8	9.2	1.5
Average....	8.6	13.6	2.3	1.0	1.4	15.0	3.8

A. "agile" isolated from Petersburg garden soil. Pellicle normally formed in the 100 cc. 2 to 2.4 per cent mannite or glucose medium used per vessel and giving a layer 2 to 3 mm. deep. Analyses after centrifuging and filtering.

nitrogen compounds occurring as normal cell constituents of Azotobacter, by normal metabolic (chiefly catabolic) processes bearing no close connection to the mechanism of nitrogen fixation.

It has been possible to collect, in tables 14 and 15, *all* of Kostytschev's pertinent experiments. It will be seen that, in the first experiments which attracted so much attention (table 14), no more than 8 per cent NH_3-N of total-N was obtained, the yields of amino-N being, in fact, usually greater than NH_3-N by a factor of two- to threefold, and the total soluble-N being never more than 20 per cent of the total-N. Winogradsky did not report values for total-N, but it would appear from probable values based on organic substrate supplied that in general he obtained about the same yields of nitrogen fixed but somewhat smaller yields of NH_3 than did Kostytschev. In 1930 (33) Winogradsky mentioned obtaining 3 to 4 mgm. NH_3-N per 2 to 2.5 gm. Na-lactate, which, with an estimated total fixation of 15 to 20 mgm. N (table 15), would correspond to 20 per cent NH_3-N of total-N; and in 1932 (34) there were reported: Experiments V, VI, VIII, and IV, 3.36, 1.68, 0.38, and 3.05 mgm. NH_3-N per 2.5, 2.5, 2 gm. Na-succinate and 10 cc. alcohol, after 6, 5, 7, and 7 days' growth.

TABLE 15

Experiments of Kostytschev and Scheloumova (21) on the formation of NH_3-N in cultures of Azotobacter grown in free nitrogen

CULTURE NO.	AGE	NET N IN CULTURE (MGM./GM. MANNITE = MGM./75 CC. GEL)			pH
		Total	NH_3-H	Per cent NH_3-N of Total-N (calc. by B. & H.)	Final
	days				
I	2	6.93	0.07	1.0	7.1
	4	10.10	0.27	2.7	7.2
	6‡	10.62	0.56	5.3	7.4
	12	9.24*	1.21	13.1 (24)†	7.7
	14	9.10*	1.23	13.6 (26)†	7.7
II	6	12.51	0.99	8.0	Not given
	8	12.44	0.78	6.2	Not given
	10	11.57	1.28	11.1	Not given
	12	12.97	1.66	13.1	Not given
III	4	6.93	0.14	2.0	Not given
	6	8.89	0.21	2.3	Not given
	8	10.01	0.42	4.4	Not given
	10	12.00	0.32	3.8	Not given
	12	12.93	1.47	11.4	Not given
IV	2	7.77	0.07	0.9	Not given
	4	9.04	0.14	1.5	Not given
	6	10.15	1.05	10.4	Not given
	8	11.83	1.36	11.6	Not given
V	4	9.85	0.63	6.4	Not given
	8	12.51	1.61	12.9	Not given
VI	6	9.65	0.98	1.0	Not given
	8	10.40	1.50	14.4	Not given
	12	10.66	2.49	23.3	Not given

A. vinelandii isolated from Krym soil. Grown in washed air on 150 cc. nitrogen-free silica gel blocks containing 2 gm. mannite, at 25°C. (III, 20°C.).

* Loss of NH_3-N by volatilization in this single experiment (in others NH_3 collected, or not lost).

† Calculated, by present writers, assuming total N = 10.62, and true NH_3-N value equal to observed value plus difference between 10.62 and observed total-N value.

‡ Kostytschev and Scheloumova remark, "The mannite was not consumed by the 6th day, and the ammonia formed during this time is therefore the first isolable product of nitrogen fixation [trans.]." It is very probable, however, that the mannite had by this time attained a very low figure. No value was measured, or cited, here or elsewhere in the paper, regarding the concentration of added organic substrate existing subsequent to addition. By general inference, some substrate remained in the blocks until at least the second to fourth day, or until the total nitrogen no longer increased, but, of course, not necessarily in all local areas of the gel. . . . Addition of sugar to cultures 4 to 6 days old resulted, by the eighth to tenth day, in 50 to 100 per cent more fixation of nitrogen, and a 60 to 80 per cent decrease in (but not total elimination of) NH_3-N.

In the great majority of the experiments, however, still smaller amounts were indicated, usually 0.01 to 0.05 mgm. of NH_3-N per culture (2 gm. substrate) per day, with a total liberation of 0.1 to 0.5 mgm. after a period of several days.

By ascertaining and then employing more optimum conditions, it was possible in the present experiments to obtain yields better than those just cited by a factor of two- to tenfold. Since it was possible to show clearly that the NH_3-N obtained even in this greater yield was not due in the slightest to a direct synthesis from N_2, it is concluded that all the NH_3-N obtained by the previous workers cited was likewise derived from cell-N. This conclusion would appear to deserve great weight since it was possible in the present investigation to repeat and confirm the findings of these previous workers when employing their technique. It cannot be urged that the results presented here are restricted in validity to the centrifuged suspensions employed in good part.

Winogradsky had advanced three reasons, all of them physiological or morphological, not chemical, for believing that the NH_3 he obtained was ordinarily derived by direct synthesis from N_2: the cultures he observed were (*a*) young (3 to 7 days), (*b*) growing, (*c*) undegraded in appearance. As a matter of fact, however, by the time NH_3-formation had commenced, the cultureswere long past the logarithmic phase of maximum growth rate characteristic of truly physiologically young cultures, and many or most of the cells were relatively quiescent. Increase in cell mass had in fact come practically to a standstill before NH_3-formation commenced, as a result of lack of organic substrate, a factor to which much less significance was attached than was merited. In regard to point *c*, we have already indicated that cells are little altered in outward appearance even after a demonstrated loss of 50 per cent of their nitrogen as NH_3-N, under conditions precluding any possible utilization of N_2 during the process of loss (H_2 replacing N_2 in the gas phase). The formation of NH_3-N by macerated and toluene-treated cells, observed by Winogradsky, is likewise readily understandable, upon a basis of results presented in this paper, as simple decomposition, aerobic or anaerobic, of cell fixed nitrogen. Our observations in regard to pasteurization and the influence of toluene show that the enzyme systems concerned in deaminative decomposition are relatively insensitive to agents which have such marked inhibiting influences on more strictly vital processes such as growth. The relatively small yields obtained by Winogradsky eliminate any necessity, from the quantitative point of view, for invoking the postulate of direct synthesis from N_2.

Kostytschev had suggested that any NH_3-N obtained before practically complete consumption of substrate, was derived by direct synthesis from N_2. It is evident from table 15 that even under this condition he had never obtained more than 5, or even 3, per cent of the total N as NH_3-N. We have shown, however, that small amounts of NH_3 may be obtained when the concentration becomes reduced to a certain low value; and in the heterogeneous medium employed by Kostytschev, substrate will fall to this value (or zero) in some local regions somewhat sooner than in others, so that Kostytschev's differentiation between primary and secondary NH_3-formation is without critical support. Furthermore, Kostytschev's belief that the presence of substrate completely prohibited deamination of added nitrogenous compounds, based on experiments with peptone and glycine only, will be shown elsewhere (14) to be entirely untenable also. Certain somewhat readily oxidizable nitrogenous compounds (e.g., arginine, asparagine, glutamate) compete successfully to some extent with added non-nitrogenous substrate and may undergo appreciable oxidation and NH_3-formation, depending upon certain very favorable conditions, chiefly of relative concentrations of nitrogenous and non-nitrogenous oxidizable substrate; and NH_3-formation from urea, which requires no oxidation, is entirely unaffected by oxidizable substrate. Such compounds might well occur sometimes in Azotobacter, in small (but only small) amounts, and also account for some of the small yields obtained in the presence of mannite by Kostytschev, in addition to the explanation of low (variable) concentration of mannite just offered.

The same foregoing quantitative considerations would also appear to eliminate the 2-phase balance proposed in scheme A. It is exceedingly difficult to conceive of an equalization in the rates of the two steps, so maintained that, under the great variety of conditions to which

Azotobacter has been subjected, merely 20 per cent or less of the nitrogen involved should appear as NH_3. This becomes particularly evident when it is realized that the rate of growth in step 2 of scheme A can be varied ten to one hundredfold by a great variety of means [oxygen pressure, temperature, pH, concentration of organic and inorganic substrates, etc. (5)], means which can scarcely be held to affect step 1 independently of step 2, but nevertheless in just the same quantitative manner. If step 1 were a simple enzymic process, as pictured, there would appear to be no reason why, *sometimes at least*, this step should not proceed at a relative rate greatly in excess of that of step 2, with the formation of 5, 10, or 100 times as much NH_3-N as cell-N, the cells acting simply as catalytic agents. The theory of 2-step balance is clearly untenable, even pictorially.

Similarly, no quantitative experiments were advanced to demonstrate the operation of the mass law in a reversible synthesis of NH_3 from N_2, as suggested in scheme B. The incorrectness of this proposition is evident from the fact that addition of NH_3 to growing Azotobacter cultures, in any concentration greater than that necessary to prevent N_2-assimilation, has never resulted in any observed liberation of N_2 (5). The well-known inhibiting action, on fixation, of added fixed nitrogen compounds such as ammonia, nitrate, and peptone, cited by Kostytschev, or others (adenine, asparagine, urea, glutamate, alanine, etc.), is readily interpretable upon a basis of preferential substrate utilization; that is, these fixed nitrogen compounds are simply more readily available. This partly kinetic, rather than purely thermodynamic, phenomenon is of widespread physiological occurrence with respect to all types of substrates, nitrogenous or otherwise. The fact that such a great variety of fixed nitrogen compounds inhibit fixation just as ammonia does, would appear to render any specific operation of the mass law in regard to NH_3 uncalled for. Finally, in our suspensions undergoing deamination, in the absence of added organic substrate, we have never observed any loss of total fixed (Kjeldahl) nitrogen in enclosed suspensions in which large amounts of NH_3 have accumulated; nor has accumulated NH_3, or added NH_3, had any measurable inhibiting action on the further formation of NH_3, up to at least 2000 mgm./l.

Proposed mechanism of NH_3-formation

The following mechanism is herewith proposed as consistent with all the findings presented in this paper and with all those obtained by previous investigators:

$$N_2 + \text{organic matter} \xrightarrow[\text{(growth)}]{\overset{1}{\text{(enzymes)}}} \text{stable cell compounds of reduced nitrogen} \underset{\text{(growth)}}{\overset{\overset{2}{\text{(deamination)}}}{\rightleftharpoons}} NH_3 \qquad (C)$$
$$3$$

NH_3, as such, is by this scheme very definitely not the first product of fixation which it might be possible to isolate. Growth, and elaboration of cell nitrogen compounds such as protein, amino acids, nucleic acid, amides, etc., occur to some (undetermined) extent by step 1, without the necessarily prior intervention of NH_3; and growth may also occur with NH_3 definitely derived in step 2 from compounds produced in step 1. Any NH_3 produced in step 2 and not utilized by step 3 in growth would appear as extracellular ammonia and account for that heretofore observed.

Scheme C does not attempt here to distinguish in step 1 between stages in growth proper, and stages in the chemical mechanism of fixation proper [involving azotase and nitrogenase (5)]. But it clearly places the formation

of any *previously observed* extracellular NH_3 *subsequent* to the formation, from N_2, of some other stable cell compound, or compounds, of reduced fixed nitrogen. The possibility of NH_3 being formed in Step 1 (with or without growth occurring) cannot, of course, be eliminated, but as already extensively indicated, no critical data in support of this are *at present* available, although the work of Bach, Yermolieva, and Stepanian (2), in which large yields of ammonia were reported with "non-living," cell-free preparations of Azotobacter, is suggestive in this connection.

TABLE 16

Formation of non-NH_3-N during the course of ammonification of cell suspensions (Burk and Horner)

TIME	DRY MATTER PER CC.	N*	TOTAL-N PER CC.	NH_3-N IN FILTRATE PER CC.		SOLUBLE N IN FILTRATE PER CC.	NON-NH_3-N PER CC.	PER CENT TOTAL N AS	
				Ness.	Dist.			NH_3-N	Non-NH_3-N
hours	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		
22	....	....	0.350	.....	0.062	0.121	0.059	18.0	16.8
46	....	....	0.340	0.085	0.100	0.175	0.075	27.2	21.9
70	1.13	15.6	0.340	0.110	0.124	0.164	0.040	34.4	11.7
91	....	....	0.318	0.135	0.143	0.167	0.024	43.8	7.6
140	....	....	0.314	0.145	0.134	0.164	0.030	44.4	9.6
15 days	0.89	13.1	0.332	0.187	0.189	0.215	0.026	56.5	7.8
25 days	0.65	17.3	0.319	0.187	0.205	0.208	0.003	61.2	0.9

* Based on total N minus total soluble N in filtrate.

4-day *A. vinelandii* culture made up into 150 cc. stock suspension containing initially 0.340 mgm. cell-N per cc., and 2.26 mgm. cell dry matter (D.M.) per cc. (15 per cent N). Incubated in organic matter-free medium at pH 7.8 and 32°C., in a closed Erlenmeyer flask. Gas phase, air. Samples withdrawn (at varying times) for analysis for total N (Kjeldahl), and then filtered through celite and analyzed for NH_3-N (Nessler's and microdistillation), total soluble-N, and non-NH_3-N (by difference).

Notes: The dry matter decreased to less than one third, whereas the total N and nitrogen percentage in the dry matter remained essentially constant, error being considered. The Nessler and microdistillation methods for determining NH_3-N gave essentially agreeing results. Initially the soluble, non-NH_3-N is almost of the same order as the NH_3-N, but eventually it all disappears, practically.

It might be thought that, *a priori*, NH_3 is *necessarily* involved to a very large extent in growth and protein formation. However, many fixed nitrogen compounds provide a faster rate of growth than NH_3, and hence it could appear that they are utilized, for the most part at least, without the nitrogen in them being first converted to NH_3-N. By the same line of reasoning, although N_2 is essentially completely reduced, it is not *strictly* necessary for NH_3 itself actually to be one such reduced compound involved. We have indicated elsewhere that some amide may possibly be specifically involved (7, 8). It would appear desirable to take the view that in fixation several successive reactions are involved, taking place in very dilute solution, the various intermediate compounds formed normally having a very brief existence and being rapidly changed into some further product; consequently, whereas the initial substances involved and to some extent the final products of the reaction may be

known, there will be comparatively little known as to the various stages in the formation of the final product, and that information will be determined probably by rather special means which will in any event require the closest analysis and circumspection.

Step 2 undoubtedly takes place, in part, through the intermediate formation of extracellularly liberated, fixed nitrogen compounds, such as have been observed by Kostytschev (table 14), Halversen (12), and Ranganathan and Norris (29). We have observed their accumulation to the extent of 10 to 75 per cent of the NH_3-N (table 16). Their actual concentration apparently increases at first, as a function of time, and then decreases, as their conversion to NH_3-N becomes nearly complete (90 per cent). Kostytschev had believed on the basis of the results given in table 14 that extracellular amino-N was formed in a synthetic process from the NH_3-N (itself derived directly from N_2), but from results of this paper it is very probable that the converse is the case, namely, the extracellular amino-N is formed in a degradation process and then by oxidation and hydrolysis further gives rise to some or most of the NH_3-N observed. This matter is being studied further.

Postscript added to galley proof. Shortly after submission of this paper to press there appeared an article by Winogradsky, "The Method in Soil Microbiology as Illustrated by Studies on Azotobacter and the Nitrifying Organisms" (*Soil Sci.* 40: 59–76, 1935). In this article Winogradsky gives an excellent characterization of the differences in outlook and method necessarily involved in studying physiological, chemical, industrial, or medical bacteriology on the one hand and soil microbiology (agrobiology) on the other. It is our opinion that the agrobiological method, so advantageous in studying the activity of Azotobacter in its natural environment, can be of but limited service in attacking the essentially biochemical problem under immediate concern here, namely, the significance of ammonia formation in its relation to the mechanism of nitrogen fixation. As indicated in detail throughout this paper, it is difficult if not impossible in the agrobiological method to carry out a number of different types of control experiments necessary in interpreting observations bearing on the "NH_3 theory" of Kostyschev and Winogradsky. Winogradsky finds the "NH_3 theory" opposed to the "Azotase theory" (5), but this is difficult to understand, since the former is concerned with intermediate products possibly involved in fixation and the latter with enzymic catalysts possibly involved. He states that the "Azotase theory" is based "on the non-production of soluble nitrogen as an essential condition," but no claim has been made as to any such necessary condition, only that it was a normal one, and in the original description of azotase (and nitrogenase) it was stated that "freedom from growth limitation, and extracellular isolation, are eventual aims" (5, p. 24). The implications of the since published work of Bach, Yermolieva, and Stepanian (2) lie in this direction, and opportunity may be taken here to state, briefly, as a result of recent direct contacts established through the very great courtesy of Academician Bach and his co-workers, that the outlook is very promising.

SUMMARY

In this paper are presented the results of a detailed and widely varied investigation concerning the chief qualitative and quantitative factors governing the extracellular production of ammonia by Azotobacter grown in free and various forms of fixed nitrogen. Both *A. vinelandii* and *A. chroococcum* have been employed, with no essential differences observed. It is shown that the extra-

cellular ammonia *observed so far, in our own and in previous investigations*, is derived in all probability entirely from decomposition of normal cell nitrogen and not, in any measurable quantity, by direct synthesis from free N_2. In this connection a quantitative critique has been given of all previous theories bearing on the origin of the ammonia, and the major previous experiments have been repeated and confirmed. As a result of *much additional experimentation*, it is shown that the view, previously advanced, that ammonia occurs in reversible (mass law) equilibrium with N_2 is untenable; and that likewise the view that the ammonia is produced from a disturbed balance between primary synthesis from N_2 and utilization in growth is also untenable. The ammonia observed has been liberated *after*, not *before*, a synthesis into cell nitrogen. The occurrence of any ammonia as an essential intermediate product in the fixation of nitrogen, *although possible, still remains to be demonstrated.* In support of these several conclusions, the following specific findings have been obtained:

Under optimum conditions Azotobacter liberates aerobically a maximum of 50 per cent of its cell nitrogen as ammonia, and anaerobically a maximum of 10 per cent. The optimum pH is 7.8 to 8.0, with a very broad, effective range exceeding the limits of pH 5 and 10. The optimum temperature is 30° to 40°C., with a very broad range exceeding 10° and 50°. The presence of N_2 gas for extensive ammonia formation is totally unnecessary once the cell nitrogen has been formed. H_2 is likewise inert. The influence of O_2 in oxidation of cell material is independent of the pressure between 0.01 to 1 atmosphere.

By far the most important factor for optimum ammonia formation is elimination of oxidizable substrate from the medium, either slowly by respiration, or quickly by mechanical separation (centrifugation). Most oxidizable organic matter inhibits the formation of ammonia completely at the low concentration of 0.03 per cent or less. Upon quick mechanical removal of substrate from the growth medium of cultures of whatever *appearance or age* (1–30 days), ammonia formation sets in immediately and, under optimum conditions, without lag in rate. The time course follows a first order (logarithmic) course, with a specific rate of decomposition of 2 per cent of the transformable cell nitrogen per hour (1 per cent of the cell nitrogen), with only about a day required to obtain half of the final, maximum possible decomposition.

The process of aerobic ammonia formation is at all times closely related to the *oxidation of cell material*, corresponding approximately to protein oxidation, since with ammonification carried to completion, the respiratory quotient (CO_2 produced/O_2 consumed) is 0.8 to 0.9, and 4.5 mols of O_2 are consumed per mol of NH_3 formed. Ammonia formation and oxidation of cell material in the absence of substrate follow the same general time courses and show a remarkable degree of correlation in relation to inhibition by a large number of agents, including *toluene*, cyanide, very high nitrate concentration, very low oxygen pressure (below 0.01 atmosphere), pasteurization, adverse pH, and adverse temperature. Inhibition by added organic matter (sugars, organic acids) may be divided into three classes: substrates themselves oxidized and inhibiting both ammonia formation and oxidation of cell material; substrates not oxidized but nevertheless inhibiting these processes; and substrates not oxidized and not inhibiting. The simultaneous inhibition of both ammonia formation and cell oxidation ordinarily occurs, for any given organic substrate, over the *same* concentration range, which, for the first substrate class, is usually 0.01 to 0.03 per cent and, for the second substrate class, 1 to 10 per cent.

The overall process of anaerobic formation of ammonia is hydrolytic, and carbon dioxide is normally not formed. Anaerobically there is no inhibition by toluene or (so far as tested)

by any of the three classes of organic substrates mentioned in connection with aerobic inhibition.

Cultures grown previously in fixed nitrogen, such as nitrate, ammonia, peptone, urea, asparagine, glutamate, adenine, creatine, or alanine, and then transferred to an environment containing *neither fixed nor free nitrogen* (the latter replaced by hydrogen gas), yield ammonia quantitatively in essentially the same manner, both aerobically and anaerobically, as cultures grown with N_2. This broad finding, in particular, shows that the mere occurrence of ammonia in cultures of Azotobacter grown in N_2 cannot be regarded as critical evidence in favor of a view current that the ammonia observed is derived, either wholly or in any part, specifically and directly from N_2.

From the agronomic point of view this paper demonstrates that a large part of the nitrogen which might be fixed by Azotobacter in soils would be liberated in a form readily available to plants, spontaneously, without the necessary intervention of other microbiological agents. By virtue of its heterogeneity the soil will provide at one time or another conditions satisfactory for the spontaneous liberation from Azotobacter cells of ammonia, and, to some extent, other soluble nitrogeneous compounds. It is likewise shown that Azotobacter cells are able to ammonify vigorously, and generally with oxidation, a large number of fixed nitrogen compounds, including many amino acids and proteins.

It is probable that the majority of findings obtained in this investigation with respect to Azotobacter would be found to be characteristic of many soil organisms, if a similar detailed study were made. The findings are in every instance consistent with known chemical, physiological, and bacteriological phenomena and should be readily repeatable.

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A PRACTICAL ANTIMONY ELECTRODE FOR SOIL pH DETERMINATION

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The antimony electrode is attractive to the soil investigator, since it offers a means for the potentiometric determination of pH without the addition of any active chemical or gas, and with equipment which may be simple and very rugged in construction, in contrast to the delicate apparatus required for the glass electrode method. Although the antimony electrode has not the unique advantage of the latter, viz., absolute immunity to disturbance from redox potentials which affect all metal electrodes, the oxidizing substances of normal soils are so insoluble that they appear to have no effect upon the antimony, however active they may be toward quinhydrone in solution.

A comparative study of methods for the determination of soil pH (4), in which data obtained by the use of the glass electrode were taken as the standard, has indicated that the antimony electrode is quite satisfactory, much superior in accuracy to the quinhydrone method and modifications thereof, as well as various colorimetric methods applied to soils, but apparently significantly inferior to the hydrogen electrode in point of accuracy. The mean of all determinations on 17 samples was pH 5.10 for the glass, 5.07 for the hydrogen, 5.24 for the quinhydrone, and 5.01 for the antimony electrode. These figures are consistent with the expected trend of errors of the several methods—the soft glass membrane *may* supply soluble alkali, streaming H_2 removes CO_2, quinhydrone reduces MnO_2 to $Mn(OH)_2$, and the technic of the antimony electrode method ensured equilibrium with the laboratory air with respect to CO_2 content.

No review of the already considerable literature on the antimony electrode in soil work will be attempted, as this has been sufficiently covered in previous contributions from this laboratory (1, 4). The theory of the antimony electrode is discussed by Roberts and Fenwick (6), who showed that under ideal conditions, including exclusion of air, the relation of potential to pH is as predicted, between pH 2 and 10, with 24 hours allowed for equilibrium. The latter requirement as well as the technic in general employed by these authors is obviously impractical for rapid measurements on soil suspensions. In application to the latter, Hooghoudt (3) compared various forms of electrodes, including the electrodeposited metal recommended by Roberts and Fenwick, and concluded that in a solution exposed to air a simple cast rod is as good as

any, and that a special addition of antimony trioxide is unnecessary. Nearly all who have worked upon soils have used the cast rod electrode, mostly without addition of oxide. In reviewing the published accounts of the behavior of the antimony electrode in buffers and soil suspensions, etc., one is struck by the fact that all recommend it for practical use and give equations for calculating indicated potentials to pH values, without agreement in the latter. The fact that the potential-pH relation of the cast antimony electrode is not a constant is generally recognized by those who have studied its practical application; the equations specified are applicable only under narrowly prescribed conditions. Snyder (7, 8), for example, specified that the potential be read while the suspension is being shaken; Hooghoudt (3) considered rotation of the electrode preferable; Barnes and Simon (1) recommend that the soil suspension be allowed to settle, and the electrode be thrust to the bottom with a reading 10 seconds later, checked by rotating the container a few times and reading after 10 seconds, repeating to constancy. The writer's experience has been that the most consistently reproducible potential differences were obtained with a suspension so heavy that it did not settle quickly, thoroughly but not violently stirred about the electrode, then allowed to rest with the electrode near the bottom for one minute before being read.

The potential assumed by an antimony electrode in a buffer or soil suspension appears to depend to a considerable extent upon the state of the electrode surface, as well as upon the pH of the medium. If the metal is tarnished or covered by a film of oxide, the potential may be very different from that shown by a highly polished electrode. Vlés and Vellinger (10) observed that a newly polished electrode placed immediately in a buffer exposed to air has a reproducible potential, which changes with time in a regular manner, whether or not the electrode is in the buffer; they conclude that the crystals of metal at the surface are placed in a state of strain by the polishing operation, from which they gradually recover with accompanying change in electrical characteristics. Vlés (9) sought to take advantage of this constant rate of change by using two electrodes with identical treatment, the one in a buffer of known pH as the standard electrode. Hooghoudt (3) reported discouraging results with this arrangement in soil work, and trials in this laboratory were no more successful. It seems probable that the factor of mechanical abrasion by soil particles may be important. The writer has noted that an electrode kept in a 1:2 suspension of a silt loam soil mechanically stirred at a rate just sufficient to prevent settling soon comes to a state of comparatively slow change in potential; if now the electrode be rotated half around, so that the side formerly protected is exposed to abrasion, the potential may change by 10 mv. or more, with slow drift as before. This effect was not noted in clear buffer solution. Rapid changes from soil suspension to buffer, both stirred in the manner described, were productive of consistent differences in potential, indicating that the change was more in the electrode than otherwise, although Hooghoudt (3) considers increase in antimony content of the solution an impor-

tant cause of drifting potential. It is difficult to reconcile this opinion with the repeated observation that an addition of antimony trioxide to the solution has no consistent effect upon electrode potentials. However, it is generally agreed that the electrode is unreliable in the presence of acids at pH values below 2 and in the presence of substances, e.g., hydroxyacids, having a specific solvent power for antimony. Citric acid, a constituent of some buffer mixtures, is of this class.

The observation that *differences* in potential shown by the rod antimony electrode in suitable buffers taken as standards and other buffers or soil suspensions were consistently near the expected values when the measurements were made in rapid sequence, suggested that it might be practical to employ the antimony electrode in a manner analogous to the accepted technic with the glass electrode, i.e., with frank recognition of the fact that the electrode has no fixed characteristic but is constantly likely to change. But whereas the characteristics of a glass film suitable for use as an "electrode" closely approximate constancy, or at least change very slowly because of inherent chemical stability, this cannot be claimed for the antimony electrode. Nevertheless, with a suitable uniform and rapid technic, experience has indicated that the change in the antimony electrode is sufficiently regular that indications of satisfactory accuracy for most soil work can be depended upon. When operating in this manner, a crucial point is whether or not the electrode is sufficiently sensitive to *change* in pH, i.e., is not sluggish or subject to lag. Best (2) has stated that this is a serious defect of both the antimony and quinhydrone electrodes, and several other authors have stated that an antimony electrode heavily tarnished is slow in response to changes. Using a reasonably clean electrode, the writer has noted no difficulty from this source. In work with soils, the slight abrasion by mineral particles seems to be sufficient to keep the surface in good condition, although after long disuse or contact with a solution causing a heavy tarnish to form, a cleaning with No. 00 sandpaper may be advisable. A newly scoured electrode is subject to rapid change, however, and the indications are likely to be less reliable than those obtained after some use.

With the foregoing in mind, a simple assembly of apparatus has been designed, enabling the use of a rod electrode in soil suspensions and buffers in rapid sequence, with agitation as desired and maximum protection of the electrode against external contacts and changes in environment. An outer mantle is made by drawing out and cutting off the closed end of a Pyrex test tube and sealing on a side arm for attachment of a rubber pipet bulb, as sketched in figure 1. By means of a perforated cork, the antimony electrode mounted in a straight glass tube is supported within the mantle, as shown. A suitable electrode is easily made by drawing the end of a 6–7 mm. i. d. Pyrex tube to a short rounded cone and cutting off to about 10 cm. length. Fragments of C. P. antimony are dropped into the open end and melted over a burner until the casting is about 3 cm. long. The tube is then set in a crucible filled with

sand in a furnace at 650–700°C., somewhat above the melting point of antimony, and allowed to cool slowly. By cautiously reheating over a burner and cooling in water, the glass may be cracked off with little risk of breaking the very brittle casting. Electrodes made thus are coarsely crystalline and, in the writer's experience, superior in performance to these suddenly cooled from the

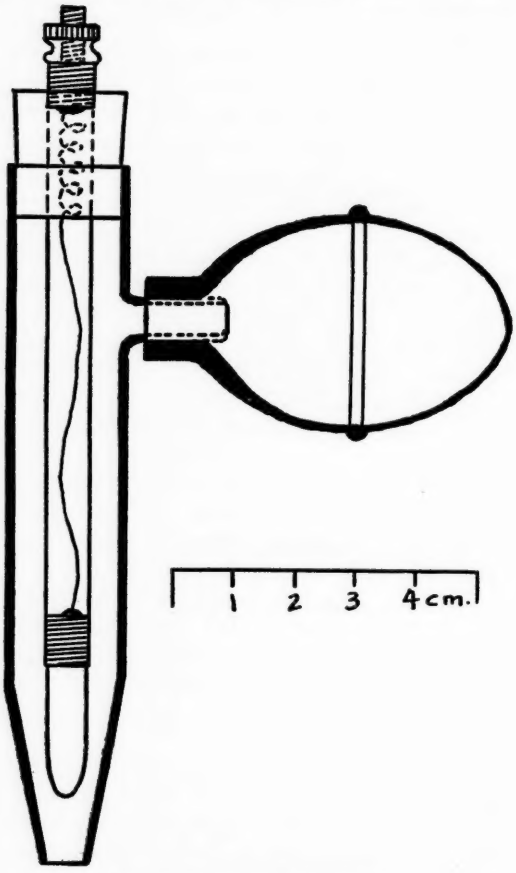

FIG. 1. ANTIMONY ELECTRODE

molten state; the latter are finely crystalline and are much less likely to break. A copper wire is carefully soldered to the antimony with ordinary solder, rosin being used for flux, and about 1 cm. of that end sanded until it will readily enter a length of the same tubing. The metal and tube are then warmed and coated with sealing wax and fitted together. A screw binding post from a discarded dry cell may be cast into a plug of solder, to which the other end of the wire is

attached, and fastened into the top of the tube with wax, for a neat external connection. The exposed surface of the antimony is sanded smooth with No. 00 sandpaper before the parts are completely assembled.

The antimony electrode is invariably connected to the negative binding post of the potentiometer. A saturated potassium chloride electrode with KCl-agar filled siphon or ground glass-plugged tube filled with crystals and solution for a salt bridge completes the cell. The sample of soil with sufficient water to make a mud easily drawn up into the mantle is contained in a 50-ml. beaker. With air-dry soils, it is well to prepare this mixture a half hour or so beforehand, as a tendency for pH to change rapidly at first is often noted. The suspension is thoroughly stirred with a rod, then drawn up into the mantle some distance above the exposed antimony, expelled and drawn up again a few times. The filled electrode vessel is finally allowed to stand with its tip in the mud remaining in the beaker. The siphon tube is then brought into contact with the latter, and the potential read one minute after the suspension was allowed to come to rest. The electrode is washed quickly by drawing up and expelling distilled water a few times, and the potential in a phosphate buffer is determined in the same way. The pH of the soil suspension is calculated by use of the formula

$$\mathrm{pH}_X = \mathrm{pH}_B - \frac{E_B - E_X}{0.000198322\ T}$$

in which the subscripts B and X refer to buffer standard and unknown respectively, E is the indicated potential in volts, and T is the absolute temperature, $273 + t°\mathrm{C}$.

The factor $0.000198322\ T$ is common to all electrode equations, indicating the effect of temperature upon the relation between differentials in potential and pH, or $dE/d\mathrm{pH}$. As numerous investigators have noted that this does not always have the theoretical or even a constant value over the entire pH range for the cast rod antimony electrode, it will probably be more accurate to standardize the electrode in *two* buffers, as closely as possible bracketing the pH of the sample. Then the observed value of $dE/d\mathrm{pH}$ may be substituted;

$$dE/d\mathrm{pH} = \frac{E_{B_1} - E_{B_2}}{\mathrm{pH}_{B_1} - \mathrm{pH}_{B_2}}, \text{ and } \mathrm{pH}_X = \mathrm{pH}_B - \frac{E_B - E_X}{dE/d\mathrm{pH}}$$

In order to avoid confusion in calculation, the buffer with numerically greater pH value is designated B_1; the other, B_2; and the soil sample, X; the corresponding potentials (E) and pH values are designated by these subscripts. Potentials expressed in decimal volt and pH values are considered positive, but the calculation is algebraic with regard to sign. This procedure has the advantage that all necessity for an uncertain temperature correction is avoided, provided there is constancy during the three measurements with the antimony electrode.

Phosphoric acid-sodium phosphate buffers suitable for standards may be prepared by mixing 0.2 M H_3PO_4 and 0.2 N NaOH and diluting. A 1.0 M

stock solution of phosphoric acid is first prepared by diluting 68.5 ml. of the C. P. reagent (85 per cent) to 1 liter, and a portion is further diluted to 0.2 *M*. An aliquot of the latter is titrated with 0.2 *N* NaOH to a clear yellow with methyl orange; the same volume will be required if the dilution is correct, and, if not, the proper dilution is calculated. To 250-ml. portions of the exactly 0.2 *M* phosphoric acid are added quantities of 0.2 *N* NaOH as in table 1, and the mixture is diluted to 1 liter. The exact pH value of each mixture is determined, conveniently by the quinhydrone method,[1] and employed in the calculation of the pH of the sample, as outlined.

TABLE 1

Phosphate buffer solutions

pH	0.2 *N* NaOH	pH	0.2 *N* NaOH
	ml.		*ml.*
2.0	100	6.0	280
3.0	225	7.0	400
4.0	250	8.0	485
5.0	255	9.0	500

TABLE 2

Data for antimony electrodes in buffers and soil suspensions

ELECTRODE NO.	POTENTIALS IN BUFFER SOLUTIONS					IN SOILS. E_X	INDICATED pH_X
	E_{B_1}	pH_{B_1}	E_{B_2}	pH_{B_2}	dE/dpH		
	volt		*volt*			*volt*	
1 (a)*	0.294	5.19	0.202	3.67	0.061	0.242	4.33
2 (b)	0.300	5.19	0.209	3.67	0.060	0.247	4.31
3 (c)	0.301	5.19	0.213	3.67	0.058	0.250	4.31
(d)	0.303	5.19	0.214	3.67	0.059	0.251	4.30
(e)	0.307	5.35	0.218	3.86	0.060	0.248	4.36
(f)	0.305	5.35	0.219	3.86	0.058	0.249	4.38

*(a) A rapidly cooled electrode, heavily tarnished when used.
(b) A similar electrode, clean.
(c) A slowly cooled electrode, clean.
(d) Same electrode, immediately after.
(e, f) Same electrode, several days later, different buffers.

The distilled water for preparing buffers and soil suspensions should be freed from CO_2 by aeration rather than by boiling, as the necessity of having as nearly as possible the same content of dissolved air in both has been emphasized by Parks and Beard (5). For this reason, a buffer solution in which molds have grown should be discarded or at least thoroughly aerated before use. The author has obtained some erratic indications from laboratory treated soils, which he is inclined to attribute to deficiency of dissolved oxygen in the sus-

[1] If the same calomel electrode and siphon tube are used, the latter should be well cleaned of traces of quinhydrone which may adhere, before being used again with the antimony electrode.

pensions, as the indicated pH values were much too high. The sensitivity of the antimony electrode to variations in oxygen content is a serious defect that may be demonstrated by the addition of a drop of dilute H_2O_2 to a buffer, greatly altering the potential of the electrode therein and often reversing its polarity with respect to the calomel electrode. Unlike the quinhydrone electrode, however, it is not appreciably affected by the insoluble oxidants of normal soils.

In illustration of results to be expected under favorable conditions, data obtained from tests on a silt loam soil and phosphate buffers are presented in table 2. Determinations on the same soil by Dr. J. A. Naftel, made under comparable conditions, indicated pH 4.32 and 4.24 for the glass electrode, 4.34 and 4.31 for the hydrogen electrode, and the author obtained 4.48 and 4.47 in determinations with the quinhydrone electrode. Colorimetric determinations, which were more consistent with this soil than is generally the case, indicated pH 4.1–4.2.

SUMMARY

A form of cast rod antimony electrode in a syringe-like protective mantle and the technic of rapid pH measurements on soil suspensions are described. The essential principle is a practically simultaneous standardization in a suitable buffer of known pH, in recognition of the fact that when used in this manner the potential-pH relation of the antimony electrode is not absolutely constant, but sensitivity to changes in pH and constancy in characteristics over short periods, even with change from one solution to another, are sufficient for determinations of satisfactory accuracy.

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A NEW METHOD FOR TESTING THE PURITY OF MARLS AND LIMESTONES[1]

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The method that is at present generally used for determining the purity of marls and limestones consists of treating a weighed amount of the materials with dilute acid and measuring by gaseometric methods the carbon dioxide evolved.

A method for determining the purity of marls and limestones, based upon an entirely different principle from the gaseometric method and having some distinct advantages over it, is here presented. This new method has for its principle the measurement of the specific gravity of calcium chloride solution formed when definite amounts of marl or limestone are treated with a definite quantity of hydrochloric acid of known strength. With a knowledge of the specific gravity of the acid and of the calcium chloride solutions formed when equal weights of pure calcite crystals and of marl or limestone are dissolved in equal quantities of acid, the purity of the marl or limestones can readily be calculated.

PROCEDURE OF METHOD

The exact procedure of the method consists first of preparing a stock solution of hydrochloric acid by mixing distilled water and concentrated HCl having a specific gravity of 1.18 at the rate 650 cc. of water to 350 cc. of acid. To every liter of the stock acid solution is added 15 cc. of amyl alcohol in order to prevent frothing when samples of limestone or marl are dissolved in it. The specific gravity of this stock acid solution is then determined by a hydrometer devised specially for this method.

For purposes of standardization, exactly 16 gm. of calcite crystals are weighed into a beaker and treated with 50 cc. of the acid solution. After the reaction is completed, the liquid is poured into a 50-cc. cylinder and its specific gravity determined.

The purity of marl and limestone is now determined by following exactly the same procedure. The only difference is that in the case of the marl and limestone there may be sediment in the liquid; and in order that this may settle out, the liquid is allowed to stand for about three minutes before its specific gravity

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is determined. It was found that any sediment present tends to settle out very rapidly, and it is not necessary to filter the liquid except in special cases. Various trials showed that the difference in specific gravity between filtered and unfiltered liquids from marls and limestones did not amount to more than 0.5 per cent of $CaCO_3$.

The procedure followed for calculating the percentage of purity from the hydrometer readings is as follows: Supposing that the corrected hydrometer reading of the acid stock solution is 10° Baumé, that of the calcite-acid product is 20° Baumé, and that of the marl-acid product is 15° Baumé. The 10° Baumé is subtracted from each of the other two readings. Then the difference in degrees Baumé for the marl-acid product is divided by the difference in degrees Baumé of the calcite-acid product, and the result is mutlipled by 100. The product is the percentage of purity of the marl.

In view of the various complications involved in the case of soils, this method is applied at present only to soils that are highly calcareous.

TABLE 1

Comparison between hydrometer method and gaseometric method for determining purity of marls and limestones

SAMPLES	GASEOMETRIC METHOD	HYDROMETER METHOD
	per cent	*per cent*
Marl 1	76.1	78.5
Marl 2	97.3	95.9
Marl 3	95.2	94.6
Marl 4 (dolomitic)	93.8	93.2
Limestone	97.6	98.1
Calcareous Soil 1	42.5	40.2
Calcareous Soil 2 (dolomitic)	30.5	30.2

For greater convenience and higher accuracy the reading of the hydrometer is taken at the surface of the liquid column.

The hydrometers that were specially devised for this method are small and are calibrated to read in 0.1° Baumé. They come in series, one reading from 5° to 10°, one from 10° to 15°, and one from 15° to 20° Baumé.

It so happens that solutions of $CaCl_2$ and $MgCl_2$ prepared from pure $CaCO_3$ or $MgCO_3$, as previously described, have practically the same specific gravities, 1.157 for the former and 1.177 for the latter. This fact makes it possible to determine the purity of dolomitic marls and limestones with little error when calculations are based on the specific gravity of $CaCl_2$ solutions.

As temperature exerts an influence on specific gravity, the temperature of the liquid is taken every time its specific gravity is measured. It was found that 1°F. makes a difference of about 0.04 graduation on the hydrometer. Since the hydrometer is calibrated at the temperature of 60°F., all hydrometer readings are reduced to this temperature, and calculations are made on this basis.

For temperatures above 60°F. the corresponding corrections are added to the hydrometer readings and for temperatures below 60°F. they are subtracted.

This method is accurate to about 0.5 per cent of $CaCO_3$. By using the strength of acid and quantity of marl or limestone already mentioned, the sensitiveness of the method is increased so that 0.1 graduation on the hydrometer is equal to about 1 per cent $CaCO_3$, and the hydrometer can be easily read to 0.05.

In table 1 is shown a comparison between the hydrometer method and the gaseometric method for determining the purity of some typical marls and limestones.[2]

An examination of the data in table 1 reveals at once the fact that the hydrometer method gives approximately the same percentage of purity of the marls and limestones tested as does the gaseometric method. The small differences shown in some cases have no significance in view of the fact that in the gaseometric method only 0.25-gm. samples were used whereas in the hydrometer method 16-gm. samples were used. Hence, if these small differences have any significance they should be in favor of the hydrometer method.

The hydrometer method appears to have at least two distinct advantages over the gaseometric method: first, in the hydrometer method a 16-gm. sample is used, whereas in the gaseometric method only from 0.25 to 2.00-gm. samples are used; secondly, where there are a large number of samples of marls and limestones to be analyzed the hydrometer method is simpler and much quicker because a large number of samples can be run at the same time depending upon the number of beakers and cylinders available, whereas in the gaseometric method only one sample at a time can be run.

SUMMARY

A new method is presented for determining the purity of marls and limestones.

The principle of this method is based upon determining the specific gravity, by means of the hydrometer, of the calcium chloride solutions formed when marls and limestones are treated with HCl.

A knowledge of the specific gravity of the acid and of the calcium chloride solution formed, when pure calcite crystals are treated with the acid, permits ready calculation of the purity of the marls and limestones.

The method was compared with the gasometric method, and both gave approximately the same results.

[2] In this comparison the writer is greatly indebted to O. B. Winter, of the Chemistry Experiment Station, who performed the gaseometric determinations.

SOIL SWELLING: I. THE SWELLING OF SOIL IN WATER CONSIDERED IN CONNECTION WITH THE PROBLEM OF SOIL STRUCTURE

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Absorption of water by soil, leading to an increase of volume, is in many cases accompanied by the very complex phenomenon of a change in soil structure. This acquires a special interest because it presents possibilities of utilizing the phenomenon of soil swelling for the elucidation of the structure problem from a new point of view and for finding methods allowing a numerical expression of the changes taking place during this swelling. The problem of the connection between the phenomenon of swelling and the structure of substance is not a new one for chemical technology and the chemistry of colloid substances.

Bütschli (2) has shown, on many examples of swollen substances studied under the microscope, that a change takes place in their structure. Interesting studies in this field have lately been carried through in which microscopic X-ray and cinematographic technique has been applied; by Hess, Trogus, et al. (7); by Katz and Mark (12) in the microscopic and roentgenoscopic study of swollen ramie fiber; by Hess and Rabinowitch (6) in a study on the swelling of different kinds of cotton fiber in which micro-cinematrographic filming was applied.

Of particular interest for soil science are the researches of Hofmann, Endell, and Wilm (3) on the swelling of an argillaceous mineral montmorillonite. This mineral is the main component of bentonite clays, found often in soils. The authors have demonstrated that in the swollen montmorillonite all the interferences, except the one with a very small angle of reflection (001), remain in the same position, whereas the latter is displaced to an extent varying with the change in the water content. The same phenomenon of "crystal swelling" has been observed by Hofmann, Frenzel, et al. (9, 10) in graphite and graphite acid.

The purpose of the present work was to study swelling in connection with the problem of soil structure. This problem is not new in soil science. It is known that the swelling of soil acts as a factor in the formation of structure because of the pressure developing during the process. On the other hand swelling causes dispersion and pulverization. As the experiments described in this paper have shown, large structural aggregates are divided into smaller ones or are definitely disintegrated into the mechanical units of which they were composed. This process becomes comprehensible if the swelling soil aggregate is considered as an osmotic cell, the inner contents of which, as the water is absorbed, gradually liquefy.

Another important circumstance that should be noted is the change in porosity after absorption of water, this change being connected with swelling and directly related to the structure of the soil. A number of investigators [e.g., Terzaghi (25), Tiulin (27, 28)] have repeatedly indicated that a change in porosity takes place in swelling soil. The quantitative

[1] The study of swelling was conducted in the laboratory of A. N. Sokolowsky, to whom I express my thanks.

side of this phenomenon, however, has remained unelucidated, though without this, many important agrotechnical problems can not be solved (e.g., non-capillary porosity or aeration).

ON SOIL SWELLING

"Swelling" is usually understood to include the capacity of certain organic and inorganic bodies to jellify after contact with certain liquids or with their vapor. This phenomenon is accompanied by an increase in the volume and weight of the swollen substance, due to absorption of the liquid, or by changes in other physical constants, e.g., viscosity, pressure, etc.

The process of absorption of a liquid by a solid does not seem to be uniform. Capillary imbibition distinctive of porous bodies must be differentiated from the phenomenon of swelling (18). Capillary imbibition presents an analogy to what is customarily called "intermicellary swelling" (considered as a capillary phenomenon), a process arising either between the micellae or on their surface. Water is attracted by the surface of crystallites, because of the bipolar action of the ions, and tends to disunite them. No change in the degree of dispersion occurs in this process.

The process of swelling is in its essence characterized by the change in properties inside the micellae which accompanies the volume increase (intramicellary swelling). Utilizing this notion, we can distinguish between the following types of swelling:

I. The swollen micella keeps its molecular bond intact. Roentgenographically, this kind of swelling finds expression in differences—though not sharp ones—of patterns. Almost all the interferences retain their positions, except those with a small angle of reflection [swelling of crystals (8)]. This type of swelling is explained by the osmotic action of the solution within the micella. The inside of the cell is transformed into a liquid, in which Brownian movement is observed (6). According to the osmotic theory a micella is a system consisting of an insoluble elastic wall and inner contents. The inner contents—the so-called "liquid phase"—are a solution of the "solid phase." This solution represents the "third component," which according to Northrop (17) causes the swelling. According to Mattson (4, 13, 14) osmotic swelling is explained by an atmosphere of dissociating ions surrounding the colloidal soil particle. Under Na saturation the number of dissociating ions reaches its maximum, and the swelling reaches its maximum as well.

II. The swelling is characterized by a complete reconstruction of the inner structure of the micella. Roentgenographically this is expressed by the appearance of a diagram of a new substance, as, for instance, was found in the swelling of ramie fibers in concentrated solutions of alkalies (12). This type of swelling is characterized by a strong increase in dispersion, influencing greatly the amount of water absorbed, because the number of water films around individual crystals rises sharply. The action of water on aggregates of crystals consists not only in their separation from one another but in the destruction of the micella itself. Between these extreme types of swelling intermediary stages can occur, the characteristic features of which are not explained as yet.

The structure of the substance exerts a complicated influence on swelling, as has been found for cellulose (7). Structure is important also in the swelling of argillaceous minerals; for instance, the crystalline network of kaolinite and of pyrophillite is less sensitive to the action of water than that of montmorillonite (3). Soil structure too seems to exercise an influence on swelling, if the structural unit is regarded as a complex of osmotic cells, the disposition of which in respect to one another is very variable.

Proceeding from the foregoing we may consider that destruction of the soil structure, as well as unsettling of the natural disposition of the aggregates, must influence swelling to a high degree. This may be evolved from the fact that pulverized soil absorbs more water than soil that has been allowed to keep its natural structure. The methods for the determination of swelling are, however, mostly based on the application of pulverized substances.

The first experiments on the determination of swelling in substances approaching soil in their structure were made by Spring (24). This author investigated the absorption of water from the geologic point of view. He used for his experiments an apparatus for measuring the pressure arising after the imbibition of liquid by a sandy or argillaceous mass. This apparatus consisted of a container covered from below by a membrane and connected at the top with a manometer. The container was filled with the mass that was to be tested, and after its immersion in water the "suction force" of the mass was determined by reading the manometer. Later this method, slightly modified, was used by Joffe and McLean (11), by Freundlich and Sachs (5), and by Shaw (21).

Mattson (14) was the first to investigate the swelling of colloids isolated from soil and saturated with Na and Ca. It was found that swelling increases with the charge of the colloids. Anderson (1) investigated the swelling of colloids isolated from different soils and saturated with Ca, Mg, K, Na, and H cations. Substitution of a cation by another that raises the charge of the colloid particles increases the swelling. According to their influence on swelling the cations rank as follows:

$$Na > K > Ca = Mg > H.$$

A review of the existing theories for the explanation of swelling may be found in Pavlov's (19) work on the swelling of cellulose.

METHOD OF INVESTIGATION

The soil samples are taken in the form of clumps or clods without disturbing the structure. This method of taking samples is preferable to the one in which a cutting cylinder or a borer is used, because in the latter case a disruption of the structure occurs, which is particularly noticeable in compact soils. Borers can be used for sampling very loose soils, with no fear of disrupting the structure, as shown in an earlier publication (23). The use of cylindric containers for the measurement of swelling by measuring the changes of volume does not eliminate certain errors because the soil column in the cylinder dilates only along its vertical axis, and this vertical dilation does not compensate horizontal swelling. Thus, depending on the orientation of soil particles in pulverized soil, the latter may show a different volume increase, as has been repeatedly observed experimentally. The cause of this is that soil swelling is an anisotropic property, i.e., it proceeds along definite axes of the crystals, as has been demonstrated by Hofmann, et al. (8).

In studying the swelling of soil lumps it is necessary to protect them from deformation upon water absorption. It was found that this could be achieved by means of a collodion film, which lets the water through under negative pressure developed by the soil and is elastic enough not to hinder the increase of volume. A 4 per cent solution of collodion proved suitable for this purpose. The more viscous sorts of collodion produce the most durable films. The durability of the film depends also on the character of the surface of the sample. Collodion films on rough samples are always more lasting than on smooth ones.

The samples, varying in weight from 17 to 60 gm., were placed on small round stands made of fine copper wire netting and covered with a sheet of filter paper. These were put into a large flat-bottomed basin, filled with distilled water to a level a little above the wire netting of the stands and covered with

a lid to prevent evaporation. Another set of samples with a coating of collodion was similarly treated.

The samples were weighed every 24 hours for the determination of the absorption of water. It was found that equilibrium was reached in a minimum of 3 days. Water adhering to the interior side of the netting and of the clods was carefully sucked away by means of filter paper. Deformed clods were excluded. The results are shown in table 1.

These experiments show that water absorption is similar in both cases, i.e., that collodion films may be used in the same way as that usually employed in swelling experiments with porous clay plates easily permeable to water. Evidently the pressure of swelling is so great that the rôle of the elastic resistance of the collodion is negligible in comparison with it.

TABLE 1

Water absorption by soil

	SOIL CLODS WITHOUT FILMS												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Air-dry weight, *gm.*	40.17	41.44	38.79	41.86	32.01	36.5	48.7	64.0	37.43	29.17	36.3	35.20	29.73
Water absorbed, *per cent.*	33.2	39.2	34.5	38.2	34.9	36.8	39.6	41.2	39.9	41.1	39.3	38.6	37.1

	SOIL CLODS COVERED WITH COLLODION FILMS									
	1	2	3	4	5	6	7	8	9	10
Air-dry weight, *gm.*	49.21	59.08	44.33	40.79	32.76	38.31	32.71	37.41	36.40	33.21
Water absorbed, *per cent.*	35.7	34.8	35.7	36.5	37.06	39.9	37.2	36.9	37.6	41.1

Average water absorption for soil clods without films:

$$M_1 = 37.97 \pm 0.7.$$

Average water absorption for soil clods covered with collodion films:

$$M_2 = 37.24 \pm 0.61.$$

The advantage of the collodion film method is that is allows samples of every form and size to be fixed without particular difficulty. The films must be, as far as possible, of uniform thickness. If one cracks in drying, the damaged places are to be covered with collodion with the aid of a small brush or a glass rod. If a sample cracks during the swelling experiment it must to discarded.

The best method for determining the volume of a swollen sample is the hydrostatic one, i.e., by weighing the sample in the air and under water. After this the sample is dried at room temperature to an air-dry condition. As the process is reversible the volume of dry soil may be determined on the same sample with the aid of mercury (22). The absorption of water is determined with precision by the difference in weight, the error not exceeding 0.01 gm. The weight of the film is taken into account.

The amount of water absorbed (K_Δ) may be expressed in percentages of the volume according to the following formula:

$$K_\Delta = \frac{\alpha}{v_\Delta} .100 \qquad (A)$$

in which α stands for the volume of absorbed water and V_Δ for that of the swollen soil.

CHANGES IN POROSITY AFTER SWELLING

The distortion of the results in the determination of capillary and non-capillary porosity is due to the changes taking place in the porosity of soil after absorption of water and the volume increase accompanying this process, as compared with the porosity of the same soil in air-dry condition. It has been repeatedly indicated that water is distributed over a greater volume of soil and that swelling is underestimated (27). But no quantitative solution of this problem had been found.

When soil absorbs water, accompanied by a swelling of its volume, porosity experiences a change. The quantitative side of this phenomenon may be expressed as follows:

The mass of the soil m remaining unchanged, its volume V, corresponding to its air-dry condition, changes in swelling to V_Δ. Hence the volume weight of swollen soil d_Δ will be:

$$d_\Delta = \frac{m}{V_\Delta} \qquad (B)$$

The changed total porosity of the swollen soil P_Δ may be expressed

$$P_\Delta = \frac{d - d_\Delta}{d} .100 \qquad (C)$$

by analogy to

$$P = \frac{d - d_1}{100} .100 \qquad (D)$$

where d is the specific weight of the solid phase of soil, d_1 the volume weight of dry soil. From equations A, B, and C it follows that if $d_1 \gtreqless d_\Delta$ the total porosity of swollen soil $P_\Delta \gtreqless P$, the total porosity of soil in its air-dry condition. Thus from formula C the change in porosity at any water content may be found: one needs only to measure the corresponding volume of the soil.

The relative change in porosity after water absorption may be found from:

$$\Delta = \frac{P_\Delta - P}{P} .100 \qquad (E)$$

If $P_\Delta = P$, water is absorbed by the soil without any change in porosity. This is observed chiefly in the case of sandy and loamy fine sandy soils. A special interest is attached to absorption of water in the case of clayey and loamy soils, if it is considered from the point of view of the structural changes arising in the soil in the course of swelling.

As mentioned, two kinds of water absorption occur. The first is capillary imbibition, in which water is absorbed by the pores of the soil or by its interparticle spaces and does not penetrate into the structural cell. No change of the degree of dispersion occurs in this case. The second case—swelling proper—is characterized by the disruption of the soil structure accompanying water absorption. This process is very complicated, but an idea of it can be formed from the following:

Soil structure consists of aggregates, the inner contents of which are chemically inactive material. It is the so-called skeleton of soil, composed of mechanical elements. The formation of aggregates is associated with the cementing properties of the soil colloids. The cause of the swelling of such a system may be represented in different ways, as follows:

1. As a result of the electrolytic dissociation of the molecules of the colloid films, the water penetrates through the film inside through the aggregates, thus causing its destruction.[2]

Thus, swelling and the breaking up of structural aggregates go hand in hand.

2. The water is attracted by the surface of the crystallites of the colloid film as a result of the bipolar action of its molecules. The electrostatic repulsion of the ultra-crystals having charges of the same sign increases the thickness of the water film surrounding the ultra-crystals and the distance between them becomes greater; this leads to the weakening of adhesion and to the breaking up of the structural cell of the soil.

The dependence and the ratio of the capillary absorbed water to the total porosity may be found by dividing (A) by (C):

$$R_\Delta = \frac{K_\Delta}{P_\Delta} .100. \qquad (F)$$

This ratio expresses capillary porosity in percentages of the total porosity, P_Δ, which may be considered as the value of the relative absorption of water. The quantity R_Δ represents the capillary porosity when the soil is saturated with water.

Absorption of an inactive (non-polar) liquid K by the same soil will be expressed by

$$K = \frac{a}{V} .100 \qquad (G)$$

where a is the volume of the inactive liquid absorbed and V is the volume of soil in air-dry condition.

[2] Compare with Ramann's "The process of swelling takes place in the presence of reversible colloids in the soil" (20, p. 327).

The quantity K expresses the capillary porosity of soil in air-dry condition, i.e., the unchanged capillary porosity of soil. The relative value of this porosity is

$$R = \frac{K}{P} .100 \qquad (H)$$

If we compare the absorption of water with that of some inactive liquid, which causes no swelling of the soil, then, according to (H), with $K_\Delta = K$ we have capillary absorption of water by the soil pores (capillary imbibition) without damage to the aggregate condition of soil. The process of capillary absorption is not, however, limited to this relation. Capillary imbibition may be accompanied by an increase in the volume of the absorbing soil. Then $K_\Delta > K$, i.e., water absorption is greater than the absorption of the inactive liquid. However, in this case the difference between K_Δ and K is only seeming. If we correct the value of the water absorption K_Δ according to the increase in the soil's volume, K_Δ becomes equal to K. The corresponding correction is

$$r = \frac{K_\Delta .Q}{100}, \qquad (I)$$

where Q is the value of the volume increase. Thus if we have

$$K_\Delta - r = K$$

we have only capillary imbibition.

The volume increase of the soil that has absorbed water is determined from V_Δ and V according to

$$Q = \frac{V_\Delta - V}{V} .100 \qquad (J)$$

In all cases, when $K_\Delta - r > K$, there is swelling, accompanied by a change in the soil structure.

The quantitative expression of these changes may be obtained from the very simple ratio

$$S = \frac{K}{K_\Delta - r} \qquad (K)$$

It follows from the ratio (K), that when $S = 1$ there is no destruction of the aggregates. In all other cases this value will be smaller the greater is the destruction. The ratio S may be used as the index of the change in the degree of dispersion arising with the absorption of water by soil. It may be regarded as a quantity characterizing the stability of the corresponding soil structure. Therefore S acquires the meaning of a "structure coefficient."

From this point of view the usually accepted notion of "swelling water" (20)

may be substituted by a new one. We may consider "swelling water" as that part of the total amount of water absorbed which causes the complex changes in the colloid condition of soil, brings about the destruction of its structure, and leads to an increase in its degree of dispersion.

The value of "swelling water" may be calculated from

$$(K_\Delta - r) - K = Wq \qquad (L)$$

i.e., on the basis of distinguishing sharply between soil swelling and the process of capillary imbibition. Therefore the value of "swelling water" characterizes the essence of the process of swelling better than the so-called "volume of swelling." It is quite possible that "swelling water" may serve as measure of the hydrophyllic properties of soil, but in this case the question remains unanswered as to which of the components is dissolved in which: the dispersion medium (water) in the dispersion phase (soil colloids), or vice versa.

EXPERIMENTAL

The liquids used in the absorption experiments were water, paraffine, machine oil, and others as shown later. Each experiment was repeated several times. For the water absorption experiments all the samples were coated with a collodion film. In the oil absorption experiments samples of loose soils only were supplied with such films, as otherwise it would be impossible to work with them without fear of their breaking up. Preliminary tests with oil absorption, similarly to those with water absorption, showed no difference between samples with or without films.

The choice of the soil samples was based partly on their textural characteristics (loamy sands and loams) and partly on their alkalinity (solonetz), because it was presumed that in dependence on these the swelling properties of soil would reveal themselves to a greater or lesser degree of sharpness.

Tables 2 to 4 show the results of these experiments.

As may be seen from tables 2 and 3 fine loamy sand chernozem shows that absorption of water produces only a slight volume increase, on the average $Q = 4.41$. If we introduce the correction for the volume increase $r = 1.37$, we obtain for $K_\Delta - r = 29.62$ and $K = 29.47$, almost equal values. Relative capillarity, i.e., the ratio of capillary porosity to total porosity, has also an equal value for the absorption of both water and oil:

$$\frac{K_\Delta}{P_\Delta} = \frac{K}{P} = 0.70$$

Swelling water $Wq = 29.62 - 29.47 = 0.15$, i.e., practically zero.

All this leads to the conclusion that in this case a purely capillary absorption of water takes place and that no structural changes occur in the soil.

By means of water and oil absorption experiments, similar relations have been found to exist in loess-like loam from the Vinnitza Experimental Station. In this soil the average of nine water absorption determinations for $Q = 12.5$,

TABLE 2

Behavior of fine loamy sand chernozem from the terrace of Volchia River with water absorption

SOIL SAMPLE	m	V	V_Δ	Q	K_Δ	P_Δ
1	21.60	14.2	14.75	3.87	30.4	45.0
2	16.03	10.7	10.91	2.80	28.6	45.0
3	15.94	10.5	10.82	3.05	31.4	44.6
4	15.60	10.3	10.82	5.65	34.0	46.1
5	13.12	8.3	8.65	4.21	33.5	43.1
6	19.42	12.1	12.72	5.62	30.4	43.1
7	14.44	9.1	9.61	5.64	31.6	43.5
8	14.09	8.9	9.31	4.61	30.3	43.5
9	11.93	7.6	7.97	4.87	28.6	43.8
				M = 4.41 ±0.34	M = 30.98 ±0.63	M = 44.2

TABLE 3

Behavior of fine loamy sand chernozem from the terrace of Volchia River with oil absorption

SOIL SAMPLE	m	V	K	P
1	22.52	14.6	29.09	42.0
2	25.90	16.6	29.55	41.6
3	20.72	14.2	32.95	45.4
4	21.55	14.6	34.30	44.6
5	20.22	13.5	31.70	43.8
6	18.01	11.1	25.96	39.3
7	16.48	10.04	25.73	40.5
			M = 29.47 ±1.65	M = 42.46

TABLE 4

Behavior of soils with benzene and isobutylic alcohol absorption

LOAMY SAND FROM THE TERRACE OF VOLCHIA RIVER WITH BENZENE ABSORPTION					LOESS-LIKE LOAM FROM THE VINNITZA EXPERIMENT STATION WITH ISOBUTYLIC ALCOHOL ABSORPTION				
Soil sample	m	V	K	P	Soil sample	m	V	K	P
1	21.60	14.2	30.10	43.10	1	16.80	10.6	35.57	42.53
2	16.03	10.7	28.00	43.80	2	15.00	9.3	33.12	41.41
3	15.94	10.5	28.60	43.10	3	18.79	11.5	33.00	41.04
4	15.60	10.3	29.13	42.40	4	19.30	12.1	34.14	42.16
5	13.12	8.3	27.40	40.84	5	20.46	12.6	36.67	41.04
			M = 28.65 ± 0.46	M = 42.65				M = 34.50	M = 41.65

$K_\Delta = 40.1$, and $P_\Delta = 49.62$. In the case of oil absorption $V = 12.5$, $k = 34.33$, and $P = 43.58$.

In comparison with loamy sand the volume increase in loess-like loam after water absorption is considerably greater and averages 12.5 per cent. After correcting this figure ($r = 5.01$) we obtain a value almost equal to the original K, the same as in the case of loamy sand: $K_\Delta - r = 35.09$ and $K = 34.33$.

Relative capillary porosity is equal here also:

$$\frac{K_\Delta}{P_\Delta} = \frac{K}{P} = 0.80.$$

Swelling water $Wq = 35.09 - 34.33 = 0.76$, i.e., practically zero, as in loamy sand. Therefore here also, in spite of the noticeable volume increase, reaching 12 per cent, only capillary absorption of water occurs.

To show that the nature of the inert liquid has no specific action on capillary absorption, parallel experiments with absorption of benzene and isobutylic alcohol were conducted. The results are shown in table 4.

These experiments show that, as could be foretold, the values of absorption proved the same for benzene and isobutylic alcohol; for instance, for loamy sand chernozem, K (oil) = 29.47 and K (benzene) = 28.65; for loess-like loam, K (oil) = 34.33 and K (isobutylic alcohol) = 34.50.

The experiments with the absorption of polar and nonpolar liquids by the soils mentioned *demonstrate with particular clearness* the fact that liquids with a different dielectric constant are absorbed by soil in equal amounts. This leads us to the conclusion that no changes connected with electrolytic dissociation of molecules take place at the interface of the phases $\frac{\text{colloid substance}}{\text{water}}$. The process of water absorption by these soils must be considered as a purely capillary phenomenon, which characterizes these soils as structureless, lacking entirely any elementary aggregate cells.

Diametrically opposite relations are found experimentally to exist in structural soils, as exemplified here by the data on (I) loamy chernozem from the terrace of Volchia River and (II) columnar alkali (solonetz) from the Khorlov district.

In I the values for water absorption were $Q = 15.30$, $K_\Delta = 42.36$, and $P_\Delta = 55.53$; for oil absorption $K = 27.17$ and $P = 47.34$.

After introducting the correction for volume increase, $r = 6.48$, we obtain for $K_\Delta - r$ (water absorption) the value of 35.88, i.e., 8.71 more than K (oil absorption). This difference constitutes the swelling water value [$Wq = (K_\Delta - r) - K = 8.71$]. This is *the water that is used up in the process of changes connected with the breaking up of the structural cell.* Specific capillary porosity for water and oil is not the same

$$\frac{K_\Delta}{P_\Delta} = 0.76; \frac{K}{P} = 0.57.$$

For water this ratio is larger, as could be expected in connection with the breaking up of aggregates, because this break-up increases the number of capillary pores, both microscopic and submicroscopic. Corresponding to this, the structure coefficient $S = 0.76$.

Similar results, but even more striking, have been obtained also with II, the columnar alkali soil. In water absorption the value for $Q = 21.66$, $K_\Delta = 40.05$, and $P_\Delta = 46.44$. In oil absorption $K = 18.22$ and $P = 33.64$.

The correction for volume increase $r = 8.67$. Then $K_\Delta - r = 31.38$, which exceeds $K = 18.22$ by 13.16, the value of swelling water. The values of the relative capillary porosities differ more than those of loamy chernozem:

$$\frac{K_\Delta}{P_\Delta} = 0.86 \text{ (water) and } \frac{K}{P} = 0.54 \text{ (oil).}$$

The structure index is here still smaller—

$$S = \frac{18.22}{31.38} = 0.58$$

And indeed columnar alkali soil differs from chernozem by a greater content of replaceable Na (its cation exchange capacity being 17.9 m.e.; Ca, 10.95 m.e.; Mg, 2.8 m.e.; and Na, 4.45 m.e.).

The results of the experiments on water and oil absorption are summarized in figure 1.

It may be seen from this graph how greatly the values of Wq ("swelling water"), $\frac{K_\Delta}{P_\Delta}$ (water), and $\frac{K}{P}$ (oil) differ from one another by the characterof the curves for structural and structureless soils. Although the values of "swelling water" Wq and "relative capillarity" $\frac{K_\Delta}{P_\Delta}$ (water) and $\frac{K}{P}$ (oil)—do not differ in structureless soils (loamy sand chernozem and loess-like loam), a sharp divergence is observed in structural ones. In the latter, capillarity as determined by water absorption always exceeds that determined by oil absorption. In this case the results of our experiments coincide with those of the experiments on the absorption of xylol, the amount of which necessary for filling up the capillary spaces is always less than that of water, as has been shown by Tiulin (27).

The characteristic feature of structureless soils is that their Wq—swelling water value—is always practically zero. For structural soils the value of Wq will be the greater the greater is the breaking up of their structure.

DETERMINATION OF NON-CAPILLARY POROSITY

It is interesting that in structureless soils—in our experiments in loamy sand chernozem and loess-like loam—for which water absorption is a purely capillary process, the difference (i.e., non-capillary porosity) between P_Δ and K_Δ and P and K has the same value (table 5):

$$n = P - K = P_\Delta - K_\Delta \qquad (M)$$

where n stands for non-capillary porosity; K for the volume of the capillaries as determined by absorption of oil or of some other inactive liquid (or what is the same thing—the capillary porosity of air-dry soil); K_Δ, for the volume of capillaries as determined by absorption of water; P, for the total porosity of air-dry soil; P_Δ, for the total porosity of soil after water absorption.

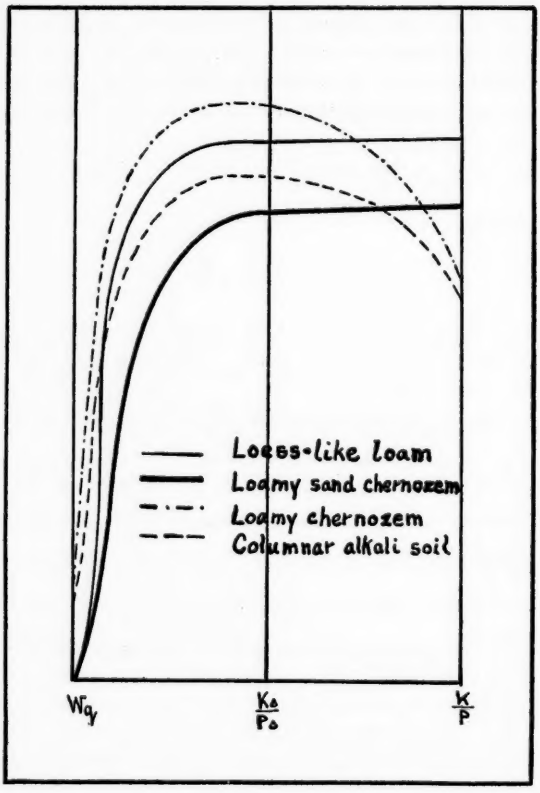

FIG. 1. "SWELLING WATER" (Wq) AND "RELATIVE CAPILLARITY"—$\frac{K_\Delta}{P_\Delta}$ (WATER); $\frac{K}{P}$ (OIL)

For structural soils the relation between capillaries and non-capillary spaces acquires another significance. It is necessary to take into account that the disruption of the structural (aggregate) cell is tied up with the dielectric constant of water. As a result of this, its penetration into the cell and the process of electrolytic dissociation associated with it will differ sharply from the penetration of a non-polar liquid. It must be assumed that the latter is absolutely incapable of penetrating into submicroscopic pores, which are accessible only

to water. Therefore, absorption of a non-polar liquid by a structural soil characterizes the "inter-particle" capillarity, i.e., the pores lying between the structural aggregates, but not the "submicroscopic" capillarity, i.e., the pores lying inside the cells. In structural soils absorption of non-polar liquids is always less, and the difference between P (total porosity) and K (oil absorption) does not represent a value characterizing non-capillary porosity. Non-capillary porosity computed in this way is greater than the real value because it includes the submicroscopic porosity. Submicroscopic pores may be calculated from formula L. They correspond to the volume of "swelling water," i.e., that water which has the property of penetrating inside the structural cell. Thus, formula M acquires the following form for structural soils:

$$n = P - K - Wq = P_\Delta - (K_\Delta - r) - Wq \quad (.12)$$

In this formula r stands for the correction for volume increase. The results are summarized in table 5.

TABLE 5

Non-capillary porosity of soils

SOIL	P_Δ	K_Δ	P	K	$P_\Delta - K_\Delta$	$P - K$	$K_\Delta - r$	Wq	$P - K - Wq$	$P_\Delta - /K_\Delta - r/ - Wq$
1. Loamy sand chernozem from Volchia River.....	44.2	30.98	42.46	29.47	*13.22*	*12.99*	—	0	—	—
2. Loess-like loam from Vinnitza Experiment Station	49.92	40.10	43.58	34.33	*9.82*	*9.25*	—	0	—	—
3. Loamy chernozem from Volchia River.....	55.53	42.36	47.34	27.17	—	—	35.88	8.71	*11.46*	*10.94*
4. Columnar alkali soil from Khorlov district.....	46.44	40.05	33.64	18.22	—	—	31.38	13.16	*2.26*	*1.90*

The possibility of determining the "submicroscopic" porosity may form the basis for a differential analysis of porosity into non-capillary porosity, capillary porosity, and submicroscopic porosity. The latter is a specific indication of the process developing at the interface of the phases $\frac{\text{colloid substance}}{\text{water}}$ and characterizes the desintegration of the structural cell.

AGGREGATE ANALYSIS, SWELLING WATER, AND INDEX OF TEXTURE STABILITY

Determinations of the amount of "waterproof" aggregates were conducted by the method of aggregate analysis elaborated by Tiulin (26). The amount of waterproof aggregates for the soils studied is compared with that already obtained for the same values of "swelling water" (Wq) and texture index (S) in table 6.

As may be seen from table 6 the results obtained by both methods coincide well. The amount of waterproof aggregates in loamy sand chernozem and in loess-like loam is practically zero, as the insignificant quantity of waterproof aggregates remaining on the sieve consists of coprolites of worms and of aggregates tied together by small roots of plants. The swelling water value for these soils is also zero, which permits classification of these soils as structureless.

TABLE 6

Aggregate analysis, swelling water (Wq), *and index* (S)

SOIL	AMOUNT OF WATER-PROOF AGGREGATES (PER CENT OF TOTAL)	*Wq*	*S*
1. Loamy sand chernozem	2.41	0.15	—
2. Loess-like loam	0.40	0.76	—
3. Loamy chernozem	28.46	8.7	0.76
4. Columnar alkali soil	12.66	13.7	0.58

TABLE 7

The influence of size and form of soil sample on oil absorption

SOIL	HEIGHT OF SAMPLE	*m*	AMOUNT OF OIL ABSORBED PER 100 CC. OF SOIL	*P*
	mm.		*gm.*	
Loamy sand chernozem from Volchia River	35.0	27.23	36.70	42.0
	60.0	45.65	37.00	42.0
	60.0	52.77	35.45	41.22
	80.0	76.27	32.60	42.0
	80.0	81.57	33.20	42.0
Loess-like loam from Vinnitza Experiment Station	35.0	19.15	33.10	43.65
	35.0	24.20	32.60	43.65
	60.0	48.68	32.50	43.65
	60.0	51.68	32.90	43.65
	90.0	94.22	30.80	43.65
	90.0	117.00	30.30	43.65

In loamy chernozem and columnar alkali soil the amount of waterproof aggregates is in inverse ratio to "swelling water" (*Wq*) and in direct ratio to the "texture index" (*S*). The greater is the value of *Wq* the less is the amount of waterproof elements. The value of the index *S* decreases with the decrease in the amount of waterproof aggregates.

SOME DETAILS OF THE ANALYTICAL METHODS AND THE SIZE OF SAMPLES

The size and form of samples used for porosity analyses is very important. Most non-polar liquids have a considerably lower capillary constant than

water. It is necessary, therefore, to pay attention to *the height of the sample* and to the character of the porosity of the soil. For finely porous soils the height of samples, it was found, must not exceed 60 mm. Experiments with saturation with oil of samples of different heights were arranged. The results are shown in table 7. Loamy sand chernozem and loess-like loam samples were taken for these experiments.

As may be seen from table 7, the sample 35 mm. in height does not differ in its absorption of oil from the sample 60 mm. high. Small deviations are due to the variations in porosity, as may be seen from the last column of the table.

Oil determinations must be executed with certain care. After absorption, the sample is left for some time on the sheet of filter paper soaked in oil. The excess of oil adhering to the surface of the sample trickles down on the paper.

TABLE 8

Influence of size of soil sample on water absorption

SOIL SAMPLE	m	V	V_Δ	Q	K_Δ	m	V	V_Δ	Q	K_Δ
1	202.02	141.41	163.08	15.68	39.63	47.70	33.33	39.08	17.25	42.09
2	196.01	136.77	156.83	14.66	41.41	40.38	28.17	32.68	16.13	42.23
3	244.55	170.64	194.58	14.02	41.39	41.49	28.95	33.70	16.64	42.31
4	215.12	150.11	175.90	16.11	43.29	38.88	27.13	31.21	15.04	45.91
5	222.18	155.03	180.77	16.60	38.58	45.37	31.65	36.38	15.00	41.18
6	236.00	164.68	191.40	16.23	40.91	46.85	32.69	37.37	14.31	41.58
7	208.14	145.23	170.78	17.58	42.37	45.37	31.65	36.47	15.22	42.09
8	238.94	166.73	191.78	15.04	39.69	37.77	26.34	30.80	16.93	41.98
				$M_1 = 15.74 \pm 0.40$	$M_1 = 40.9 \pm 0.54$				$M_2 = 15.82 \pm 0.38$	$M_2 = 42.42 \pm 0.51$

The data on swelling and water absorption for larger and smaller samples given in table 8 show the influence of the size of sample on the accuracy of the determinations.

From comparison of the average values of Q (volume increase), it follows that: $M_2 - M_1 = 0.08 \pm 0.17$. From comparison of K_Δ (water absorption), $M_2 - M_1 = 1.51 \pm 0.74$. Thus no differences have been observed. A certain increase in the water absorption (K_Δ) and swelling (Q) values is noticeable in smaller samples. This is in accordance with the "Bodenkörperregel" theory and with the experiments on the influence of the mass of swelling gelatine on its volume (13).

SUMMARY

The study of the phenomena of swelling of soil that keeps its natural structural condition allows investigation of the problem of soil structure from a new point of view.

A method has been elaborated, and a form for a quantitative expression of the porosity changes caused by absorption of water by soil, evolved.

Definitions of the "index of texture stability" (S) and of "swelling water" (Wq), founded on the sharp difference between capillary imbibition and swelling proper, are introduced.

Swelling water may be considered as the form of expressing the hydrophillic property of soil.

Soil swelling is considered as a process of changes arising in the properties of soil at the interface $\frac{\text{soil colloids}}{\text{water}}$. This process leads to a change in the structure of soil and to an increase in its degree of dispersion.

A quantitative expression of this process is possible in the form of "swelling water," which represents a more characteristic indicator of swelling than the increase of volume.

A method is proposed for a differential analysis of soil porosity, providing for its subdivision into non-capillary, capillary, and submicroscopic porosity.

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BOOK REVIEWS

Weeds. By WALTER CONRAD MUENSCHER. The Macmillan Company, New York, 1935. Pp. xxi + 577, figs. 123, tables 11. Price $6.00.

The channels of trade are also in part those of weed distribution, and, since the coming of the white man to North America, many new species of plants have been brought in. Some of them are listed among the most valuable of our staple crops. Inadvertently, there came also plants objectionable or undesirable, and they are commonly designated as *weeds*. The present work is a valuable treatise on the subject of weeds. It sums up our present-day knowledge and offers to the student and the general reader information in very accessible form.

As is noted in the preface: "Part I is devoted to a consideration of those characteristics and habits of weeds by which they affect other plants or interfere with man's activities, and also to the methods employed for their eradication and control. In Part II is brought together data concerning the names, duration, reproduction, dissemination, habitat, range, source, recognition and control of the commonest weeds of the northern United States and Canada. Particular emphasis has been given to identification and control."

There are four chapters in Part I, dealing, respectively, with the dissemination and importance of weeds, weeds of special habitats, the control of weeds, and chemical weed control. Part II contains a list of weeds arranged according to family, together with key. A key to the groups and species of weeds is also given.

Altogether, the author has succeeded in rendering a distinct service to the farmer and to the specialist in commerce and agricultural science, on whom he must depend for the profitable conduct of his business.

Suppression of Weeds by Fertilizers and Chemicals. By H. C. LONG. H. C. Long, Hook, England, 1934. Price 2s. Pp. 57, figs. 12.

The preparation of this booklet was prompted by the need for a popular presentation of the status of weed-control by means of fertilizers and chemicals. As is noted in the foreword: "For many years now the destruction of weeds by the application of some chemical has been occupying the attention of investigators, and one material after another has been recommended for practical use. Little by little information has accumulated, and as some of the methods have proved their value the time is opportune for their serious consideration by farmers." The foreword by Sir Daniel Hall and the author's preface are followed by eleven chapters, designated as follows: Losses due to Weed Infestation; Effect of Fertilizers in Reducing Weeds; The Use of Lime; Sulphate of

Ammonia and Nitrate of Soda; Calcium Cyanamide; Sulphuric Acid; Sulphate of Copper and Iron; Finely Powdered Kainit; Sodium and Potassium Chlorates; Compounds of Arsenic; and Miscellaneous. There is also a list of weed names.

The Algae and Their Life Relations. By JOSEPHINE E. TILDEN. The University of Minnesota Press, Minneapolis, Minnesota, 1935. Pp. xii + 550, figs. 257. Price $5.00.

The author is to be commended for the effective treatment of a most interesting subject. She has placed under obligation to herself many teachers, research workers, and students in the plant and soil sciences. As is indicated by the author:

> The book is primarily a text for advanced students in botany. It is designed as a guide to the student, a supplement to lectures and laboratory work, and a reference book for experienced investigators. The algal plants are described in the earlier chapters. Their relations to other plants and animals, their distribution and economic uses, are discussed in later sections.
>
> Every aspect of phycology is given attention. Terms have been simplified and reduced in number, and a helpful series of life cycle diagrams has been included. The volume also contains a discussion of the economic significance of algae; their uses as food for men and animals, their effects on water supply, and their value as fertilizer.

Der Einfluss der Düngung auf den Pflanzenbestand des Dauergrünlandes. By K. PLOTZE. Verlagsgesellschaft für Ackerbau M. B. H., Berlin, Germany, 1935. Pp. 80.

The growing interest in erosion control and soil conservation has forced the recognition upon us that the best method of controlling erosion must be based on the maintenance of a proper plant cover. Many million acres of agricultural land have been damaged because of the removal of the topsoil by way of gullying or sheet erosion. It is more widely recognized now than heretofore that, because of its topography, much of our present crop land must be maintained under a forest or grass cover if the surface soil is to be protected from further injury by erosion. Quite logically, the increasing acreage under grass almost automatically compels us to inqure concerning methods of grassland management. The author of this book discusses facts of interest to farmers, investigators, and teachers.

The book was written from the point of view of the needs of German agriculture. The author notes in his preface that Germany is compelled to increase her resources of forage crops in order that it may be less dependent on imports of certain agricultural commodities. The subjects which he deals with, in a rather popular way, consist of a general statement, the influence of lime on the botanical composition of permanent meadows, the influence of farmyard manure, the influence of potassium and phosphorus fertilization, the influence of kainit and calcium cyanamid for the control of weeds, and the influence of nitrogen fertilizer. The list of references consists of 131 titles.

Fertilization of the Canadian Prairies. The Consolidated Mining and Smelting Company of Canada, Ltd., Calgary, Canada. Pp. 48.

The booklet is made up of a number of short articles dealing with one or another phase of Canadian prairies and their fertilization. It is stated in the foreword that

> Successful fertilization of the Canadian prairie soils began four years ago, when the combined fertilizer project between the Dominion and Provincial Governments and leading industrial concerns was instigated and phosphate fertilizers were first applied by drilling in with the seed of cereal crops.
>
> This booklet contains statements from many of the leading investigators in the Dominion and Provincial Governments, Universities and Agricultural Colleges, who were closely allied with the prosecution of the project. Also a few letters from some of the many actual farm users, together with certain economic data from actual large farmer operators.

Die Bindung der Phosphorsäure im Erdboden. II. Untersuchungen aus Westnorwegen. By TORBJØRN GAARDER and O. GRAHL-NIELSEN. A. S. John Griegs Boktrykkeri, Bergen, Norway, 1935. Pp. 107, figs. 34.

This is the second part of a report on the fixation of phosphoric acid in soils. The first part appeared under the same title at Bergen, Norway, in 1930. It dealt specifically with the solubility of phosphoric acid in water solutions, with electrolytes with varying pH value and cation content.

The present volume records investigations made in Western Norway. The contents of the publication are arranged under the headings: Introduction; Discussion of the Soil Samples Investigated; Analytical Methods Employed; Discussion. There is also a résumé in Norwegian.

Handbuch der Ernährung der landwirtschaftlichen Nutpflanzen. By J. BECKER-DILLINGEN. Paul Parey, Berlin, 1934. Pp. VIII + 523, figs. 124, plates 11. Price RM 19.60.

In preparing this book, the author wished to provide helpful data not only for the practical farmer, but also for the teacher and student. The major divisions of the book are designated, respectively, "General Considerations of Plant Nutrition," "The Soil as a Culture Medium of Plants," "Fertilizers and Fertilization," and "The Fertilization of Certain Economic Plants."

Consideration is given by the author in the first part to such questions as seed germination, the synthesis of plant products, and the destructive metabolism in plants. In the second section of the book the author deals with soils and soil science, soil formation, soil physics, soil chemistry, soil biology, and soil classification. In the third part consideration is given to animal manures and organic waste products. The fourth section treats of the fertilization of cereals, legumes, root crops, oil-bearing plants, fiber crops, commercial crops like tobacco and hops, forage crops, and meadows and forests. Numerous foot notes, adequate illustrations, and a satisfactory index add to the usefulness of the book.

Der Speisewert der Kartoffel. By KARL RATHSACK. Verlagsgesellschaft für Ackerbau M. B. H., Berlin, Germany, 1935. Pp. 139, figs. 19, tables 49.

The growing of potatoes in Germany and in some of the other European countries is intended to cover a wider range of use than is true of the United States. As the author notes in his preface: "We differentiate our potato varieties in accordance with their particular fitness for manufacturing, human food, animal food or general purposes."

Since this treatise is devoted to the edible qualities of potatoes, emphasis is laid on cooking qualities, the firmness of the tubers and cooking quality as related to chemical composition, the relation of cooking quality to the length of the storage period, the changes which occur in the cooking of potatoes, the distribution of the chemical constituents within the tuber, and the chemical composition and taste. There are a list of references containing 96 titles and an author index.

Pflanzliche Konstitutionslehre. By F. MERKENSCHLAGER and M. KLINKOWSKI. Paul Parey, Berlin, Germany, 1933. Pp. 74, illus. 85. Price RM 7.50.

The authors approach their subject largely from the point of view of the ecologist. As is indicated by the table of contents, consideration is given to plants derived from humid environments and those derived from arid environments. In the first group are included potatoes, serradella, oats, and buckwheat. There are crops of a transient character, such as lupines and rye. The plants originating in arid environments include mustard, sugar beets, wheat, alfalfa, and barley.

The pamphlet is well illustrated, and the subject matter is presented in an interesting way. There is a bibliography attached to each of the major groups discussed.

Die quantitative Spektralanalyse der Elemente. By HENRIK LUNDEGÄRDH. Gustav Fischer, Jena, Germany, 1929 and 1934. Part I, pp. vi + 155, illus. 43, tables 13. Price RM 13.50. Part II, pp. viii + 124, illus. 39, tables 3. Price RM 20.

The first part of the report was published in 1929, and the second, five years later. As stated by the author, in the first part of the report, the quantitative methods for the spectrum analysis of elements were considered in their application to biological, agricultural, chemical, and mineralogical problems. It is noted in the second part that the author has improved the methods and indicated practical procedures for the carrying out of the analyses in the fields of biology, medicine, agricultural chemistry, and mining.

The contents of the first part of the report are indicated under the topics: Introduction; Quantitative spectrum analysis as based on the flame spectrum; Quantitative analysis as based on the spark spectrum; and Views on the application of quantitative spectrum analysis of the elements.

In the second part of the treatise, the major topics dealt with are designated

as Index; Quantitative emissions of spectography, and the photoelectric measurements of spectrum lines, gas analysis, practical suggestions.

The work is splendidly illustrated, and the subject matter is well arranged. The author has made a substantial and valuable contribution on the subject of the quantitative aspects of spectrum analysis.

Popoff's Quantitative Analysis Third Edition. Revised by MURRAY J. RICE and WARREN P. CORTELYOU. P. Blakiston's Son & Co., Inc., Philadelphia, Pa., 1935. Pp. xxii + 555, figs. 75.

Extensive changes have been made in the book for the sake of bringing it in line with present-day studies of quantitative analysis. In the preface to the third edition, we are told that "One who is familiar with the second edition of this book may feel, on first consideration, that little of the original material has been preserved. A detailed examination will show otherwise. Dr. Popoff's original idea that 'quantitative analysis affords an excellent approach to theoretical chemistry and to skill and precision in experimental work' has been preserved and, it is hoped, emphasized. However, much of the material has been rewritten and nearly all of it rearranged in the belief that the new presentation will simplify the work of the student and of the teacher."

The preface to the third edition is followed by that to the first edition, and this, in turn, is followed by a foreword to the instructor and a foreword to the student. Part I of Book I contains 8 chapters; Part II, 7 chapters; and Part III, 9 chapters. Book II is made up of two parts, containing, respectively, 8 and 12 chapters. The appendix includes a glossary and table of abbreviations; references to analytical chemistry; how to use logarithms; how to use cologarithms; answers to problems; antilogarithms; four-place logarithms table.

Teachers and students will both find it to their interest to examine the book and to utilize it as a valuable addition to other teaching material in quantitative analysis.

Annual Review of Biochemistry. Vol. IV. Edited by JAMES MURRAY LUCK. Annual Review of Biochemistry, Ltd., Stanford University P.O., California, 1935. Pp. vii + 639. Price $5.00.

The rapidly increasing number of workers in the general field of biochemistry and their efforts to keep themselves informed on the progress in their chosen field make the *Annual Review of Biochemistry* very helpful. In the preface to the present volume, the Editorial Committee notes: "In the introductions to the three preceding volumes we have described the circumstances leading to the inception of the *Review* and the considerations which seemed of major importance in the development of editorial policy. Most noteworthy are the unabated growth in the field as a whole, the ever-rising tide of published work urgently in need of integration and synthesis, the dramatic suddenness with which new discoveries illuminate old and vexing problems, and the tendency of other once-active phases to become quiescent."

They note, further: "In accordance with the policy of giving due recognition to fields of immediate interest, we are glad to include within the present volume reviews on choline and allied compounds by Professor Gaddum, on the biochemistry of malignant diseases by Mrs. Holmes, and on plant hormones by Professor Thimann. Reviews on soil bacteriology, biochemistry in relation to selected problems in medicine, and on the application of X-ray methods to the elucidation of the structure of compounds of biochemical interest will appear in Volume V (1936)."

The topics dealt with in the Review include: "Permeability" by M. H. Jacobs; "Biological Oxidations and Reductions" by R. Sonderhoff; "Enzymes" by J. B. Sumner; "The Chemistry of the Carbohydrates and the Glycosides" by J. C. Irvine and G. J. Robertson; "The Chemistry of the Acyclic Constituents of Natural Fats and Oils" by E. Chargaff; "The Chemistry of the Proteins and Amino Acids" by E. J. Cohn; "The Chemistry and Metabolism of the Compounds of Sulfur" by H. B. Lewis; "The Chemistry and Metabolism of the Nucleic Acids, Purines and Pyrimidines" by L. R. Cerecedo; "Carbohydrate Metabolism" by C. F. Cori and G. T. Cori; "Fat Metabolism" by C. Artom; "The Metabolism of Amino Acids and Proteins" by Y. Kotake; "The Metabolism of Creatine and Creatinine" by W. C. Rose; "Detoxication Mechanisms" by B. Harrow and C. P. Sherwin; "The Hormones" by B. A. Houssay, V. Deulofeu, and A. D. Marenzi; "Choline and Allied Substances" by J. H. Gaddum; "Vitamins" by L. J. Harris; "Nutrition" by S. Brody; "The Chemistry of Muscle" by P. Eggleton; "The Metabolism of Brain and Nerve" by E. G. Holmes; "Chemical Embryology" by J. Needham; "The Biochemistry of Malignant Disease" by B. Holmes; "Plant Pigments" by R. Kuhn; "The Alkaloids" by R. Robinson; "The Mineral Nutrition of Plants" by F. C. Steward; "Growth Substances in Plants" by K. V. Thimann; "Immunochemistry" by M. Heidelberger; "The Chemistry of Bacteria" by M. Stephenson; and "Index."

Soils of Northern and Northwestern China. By JAMES THORP and K. C. HOU. Soil Bulletin No. 12, The National Geological Survey of China, Peiping, China, 1935. Pp. 154.

Much progress has been made within the past five years in the classification and mapping of the soils of China. The senior author, on leave from the Bureau of Chemistry and Soils of the United States Department of Agriculture, has helped the Chinese students to utilize the methods developed and perfected in the United States for the classification and mapping of soils. The late Dr. C. F. Marbut, Chief of Soil Classification and Mapping of the Bureau of Chemistry and Soils, was at the time of his death at Harbin on his way to China, after the sessions of the Third International Congress of Soil Science at Oxford, to join his fellow worker. His death is a loss to soil science.

The present treatise is made up of seven chapters, entitled, respectively: "Areas Investigated," "Soils and Crops," "Soils and Their Interpretation,"

"Agricultural Industries," "Irrigation, Alkali and Drainage," "General Recommendations," and "Soil Erosion and its Control." There is also a foreword, a summary, and a list of references and notes on the soils of the Salachi Irrigation Project in relation to reclamation.

The material is well arranged, and the authors are to be particularly commended for the quality of the maps, the illustrations, and the list of references. This report is particularly valuable and significant at this time in view of the more intimate coöperation among members of the International Society of Soil Science toward the completing of an authoritative map of the great soil regions of the world.

The Struggle for Existence. By G. F. GAUSE. The Williams & Wilkins Company, Baltimore, Md., 1934. Pp. ix + 163, figs. 41, tables 9.

In his foreword to the book, Dr. Raymond Pearl, of Johns Hopkins University, says: "The author of the present treatise, Dr. G. F. Gause . . . makes in this book an important contribution to the literature of evolution." Elsewhere, Dr. Pearl notes the growing interest in the subject. "This renewed and effective activity," he says, "seems to be due primarily to two things: first, the recrudescence of general interest in the problems of population, with the accompanying recognition that population problems are basically biological problems; and, second, the realization that the struggle for existence and natural selection are matters concerning the *dynamics of populations*, birth rates, death rates, interactions of mixed populations, etc."

Dr. Pearl notes again that the author "breaks new ground in this book. It will cause discussion, and some will disagree with its methods and conclusions, but no biologist who desires to know what the pioneers on the frontiers of knowledge are doing and thinking can afford not to read it."

The six chapters in the book deal, respectively, with "The Problem," "The Struggle for Existence in Natural Conditions," "The Struggle for Existence from the Point of View of the Mathematicians," "On the Mechanism of Competition on Yeast Cells," "Competition for Common Food in Protozoa," and "The Destruction of One Species by Another." There are two appendixes, a bibliography of 138 titles, and an index.

Peasant Europe. By. H. HESSELL TILTMAN. Jarrolds Publishers, London, 1934. Pp. 282, illus. 47, map 1.

The keynote struck by the author in his foreword is a rather striking one. He says: "Western Europe, preoccupied with the problems of international relations, industry, and the future of armaments, is sometimes in danger of overlooking the fact that more than half the entire population of that Continent is composed of peasants. The immense territories of this hundred millions of cultivators (outside the frontiers of the U. S. S. R.), whose bent backs till the soil of the ocean of peasant-lands, stretch from the Black Sea to the Baltic, forming a natural barrier between East and West. The peoples who inhabit

that land of farmsteads—Poles, Ukrainians, Czechs, Slovaks, Hungarians, Southern Slavs, and the rest—together represent the largest single unit in Europe, split by artificial political walls, but united by the bonds of common interests and, in war or peace, usually a common fate. Those peasant territories remain to-day almost virgin soil for the world's manufacturers, populated by millions of potential customers clad in home-made clothing and living on the produce of their soil."

The contents of the book include a foreword and 18 chapters, designated as follows: I. The Other Half of Europe; II. Austria: Gateway to the Peasant Lands; III. The Kingdom of Serbs, Croats and Slovenes; IV. Croatia's Fight for Justice; V. The New Bulgaria Emerges; VI. Bulgaria To-day; VII. Bulgarian Interlude; VIII. Will "Greater Rumania" Achieve Greatness?; IX. Bessarabia: A Study in Decay; X. Bukovina—and its Peoples; XI. A Peasant Speaks; XII. Hungary: A Nation with a Grievance; XIII. Poland and its Peasants; XIV. A Nation Nobody Knows; XV. The Ukrainians Live On; XVI. Czechoslovakia: A Successful Experiment; XVII. The World Depression and the Peasant; XVIII. The Peasants Look at the Future; and Index.

The book makes interesting reading and is to be commended to the general reader as well as to the statesman and economist.

The Agricultural Fair. By WAYNE CALDWELL NEELY. Columbia University Press, New York, N. Y., 1935. Pp. xxii + 313, illus. 9.

To one familiar with the evolution of American agriculture, there is much of compelling interest in *The Agricultural Fair*. The organization and the procedures of agricultural fairs are a part of the inheritance from the Old World. The agricultural fair still serves as an important educational factor in this and other countries. As is noted by the editors' foreword: "No institution, perhaps, has exerted greater influence upon American rural life than the agricultural fair. Yet the story of its Old-World origin, its transfer to this side of the Atlantic, and its many-sided development in the United States has long awaited the pen of some one who was competent to trace its history and to evaluate its social significance. Fortunately Professor Neely, who essayed this double task, was admirably fitted by temperament and by training to undertake it."

Aside from the editors' foreword and the preface, there are four parts, dealing, respectively, with "Early History and Types of Fairs"; "The Evolution of the Agricultural Fair in America"; "The Functional Aspects of the Agricultural Fair"; and "Conclusion." The book contains also an extensive bibliography and an index.

Plants and Human Economics. By RONALD GOOD. Cambridge University Press, London, 1933. Pp. xii + 202, maps 8. Price $1.75.

The following may be noted from the preface: "My object," the author says, "has therefore been to combine in small compass and, it is hoped, in

reasonable and readable fashion, not only the botanical facts but also, and frequently more important, the historical and economic facts required to give to those who begin the scientific study of botany an adequate humanistic background of reality to their subject. I have tried to give them the evidence that the science of plants is something more than a mere mental discipline."

The thirteen chapters in the book are designated, respectively, I. Introductory; II. The Nature and Sources of Food; III. The Life of the Green Plant; IV. Factors limiting Agricultural Production; V. Science and Agriculture; VI. Cereals and Pulses; VII. Vegetables: Salad Plants: Fruits; VIII. Beverages: Sugar and Starch: Oils and Fats: Spices; IX. Timber, Coal and Petroleum; X. Rubber: Resins, Balsams and Gums: Tans and Dyes: Fibres; XI. Alcohol: Drugs: Fodders: Miscellaneous; XII. The Useful Products of the Lower Plants: Concluding Notes on Vegetable Products; XIII. The Economic Botany of Great Britain. The reader will find at the end of the book a selected reference list, an appendix containing scientific and English names of commercial plants arranged in systematic order, and an index.

World Sugar Production and Consumption. By C. J. ROBERTSON. John Bale, Sons & Danielsson, Ltd., London, 1934. Pp. vi + 142. Price 5s.

Sugar is one of the major human foods. Sugar cane and sugar beets—to say nothing of other plant species—are the sources of this important carbohydrate. The primary and secondary products of our sugar crops are of signal importance in their effect on the economic, social, and even political structure of many peoples.

The purpose of the author in preparing the book is explained in the preface. He says: "Exhaustive treatises on the technical aspects of sugar production have been written, dealing with cane or beet agriculture, with the factory processes in the production of raw sugar from cane or beet and with sugar refining. A book giving a comprehensive survey of the world's production of sugar from both cane and beet and with emphasis on the economic-geographical aspects has, however, been lacking."

The book contains thirteen chapters, designated as follows: I. An Outline of the World Trade in Sugar; II. General Conditions of Cane-Sugar Production; III. General Conditions of Beet-Sugar Production; IV. Cuba; V. Java; VI. India; VII. British Empire Producers Excluding India; VIII. United States Producers Including the Philippines; IX. Other Cane-Sugar Producing Countries; X. Europe and the Soviet Union; XI. The Refining Industry; XII. The Component Factors on Consumption; XIII. Prospects of Consumption and Production. There are also a bibliographical note and an index.

Fifty-Two Years of Research, Observation and Publication 1877–1929. By HENRY FAIRFIELD OSBORN. Charles Scribner's Sons, New York, 1930. Pp. xii + 160.

The author notes in his preface:

> In these days of intensive specialization both in education and in research, it is interesting to review the activities of a half-century initiated in the older, broader and more extensive school and continued in the modern, narrower and more intensive school. It has been a great opportunity and privilege to receive inspiration in the spirit of one century and to be carried forward into the grander opportunities of another century.
>
> One object of this volume is to show the *educational* value of the older school, chiefly in *fitting the mind to attack new problems from the old, broad point of view*. . . . Another object is to show that the broad education and the broad point of view never carry us beyond the lecture platform or beyond the reputation of learning and culture unless breadth is the useful threshold for depth. In other words, intensive research through thousands of intelligently directed observations enlightened from time to time by generalizations or hypotheses becomes the starting point for entirely new chains of observation purposely directed to convert a working hypothesis into a new principle or a new law.

The major divisions of the work deal with the publication of research, ideal methods of research, fellowship of research, research in the universities, and the impulse of research in geology and biology.

The reader will find much interest in this little book and will be impressed by the wide range of subjects dealt with by the author.

Report for 1934 Rothamsted Experimental Station. Harpenden, England. Gibbs & Bamforth, Ltd., St. Albans, England. Pp. 259.

Every well-informed student of soils knows something about the contributions that have been made by the Rothamsted Experimental Station in the domain of soil and plant science. The present report is a most interesting record of progress. It contains information on the organization and staff of the station, the Woburn Experimental Farm, the Imperial Bureau of Soil Science, publications of the Rothamsted Experimental Station, and other matters of interest.

The director of the station, Sir John Russell, notes the additions to the physical resources of the institution and summarizes in condensed form the accomplishments in the various fields of research with which the station concerns itself. There are brief reports by the members of the staff responsible for major research projects or fields of activity. A list of publications and brief abstracts of these publications are given. Numerous tables and other statistical data are given which supplement those in earlier reports. The reader will be impressed with the wide range of scientific thought and interest shown by the director and his associates at Rothamsted.

Transactions of the Third International Congress of Soil Science. Vol. I. Thomas Murby & Co., London, 1935. Pp. xii + 428.

The editorial committee notes in the preface that: "The papers are arranged primarily in order of Commissions—papers for Commission I preceding those for Commission II, and so on. For this purpose, the sub-Commission for Alkali Soils has been called Va, the sub-Commission for Forest Soils Vb, and

the sub-Commission for Peat Soils VIa. Within each Commission, the papers are grouped according to the different subjects for discussion, and within each group, alphabetically according to the authors' names. The groups of papers are arranged in the order in which they will be discussed by each Commission. Papers to be presented at joint sessions of two or more Commissions have been allocated to the Commissions to which they seemed most appropriate."

This volume of Transactions was made available for distribution prior to the opening of the sessions of the Congress at Oxford on July 29, and was used by the delegates and visitors at the Congress to facilitate their following the papers presented at the different sessions. The editorial committee is to be complimented for having done their work well.

The papers are grouped under the major topics. These are—Commission I. Soil Physics: "Water in Soil"; "Binding and Disruption Forces in Soil Structure"; "Mechanical Analysis and Field Texture"; and "Surface Phenomena in Soils." Commission II. Soil Chemistry: "Exchangeable Bases"; "The Soil Absorbing Complex"; "Method of Estimating Plant Nutrients in Soil"; "Analytical Methods"; and "Appendices." Commission III. Soil Microbiology: "Recent Ideas Concerning the Oxidation of Ammonia in Nature"; "The Physiology and Ecology of Nitrogen-Fixing Organisms"; "The Decomposition of Plant Materials and Soil Organic Matter"; "Methods of Estimating Plant Nutrients in Soil"; "Quantitative Investigations of the Microbiological Population of the Soil"; and "Miscellaneous." Commission IV. Soil Fertility: "Problems in Crop Production"; "The Nitrogen Supply of Crops"; "The Decomposition of Plant Materials and Soil Organic Matter"; "Methods of Estimating Plant Nutrients in Soil"; "The Importance of Deeper Horizons in Plant Nutrition"; and "Miscellaneous." Commission V. Soil Genesis, Morphology and Cartography: "Reports on Soil Maps"; "Principles and Methods of Land Classification"; "Soil Formation and Soil Types"; "The Composition and Classification of Soils"; and "Schemes of Soil Classification." Sub-Commission Va—Alkali Soils. Sub-Commission Vb—Forest Soils. Commission VI—Application of Soil Science to Land Amelioration: "Water in Soils"; "Drainage Investigations"; "Irrigation, Erosion"; and "The Movement of Salts in Soil." Sub-Commission VIa—Peat Soils.

Transactions of the Third International Congress of Soil Science. Vol. II. Thomas Murby & Co., London, 1935. Pp. 194.

This volume, containing the plenary session papers and the presidential address, was made available prior to the adjournment of the sessions at Oxford. The title of Sir John Russell's presidential address was "The Place of Soil Science in Agriculture." The volume also contains papers not included in Volume I of the Transactions. There are three papers listed under Soil Physics; two, under Soil Chemistry; two, under Soil Microbiology; three under Soil Fertility; three under Soil Genesis, Morphology and Cartography; and two under Application of Soil Science to Land Amelioration.

Report of the Iowa Twenty-Five Year Conservation Plan. By JACOB L. CRANE, JR., and GEORGE WHEELER OLCUTT. Publication of The Iowa Board of Conservation and The Iowa Fish and Game Commission, 1933. Pp. xiii + 176, plates 53.

This report was sponsored jointly by the Iowa Board of Conservation and the Iowa Fish and Game Commission. The following is quoted from the foreword:

> We believe that this report records the desire of the people of Iowa to exercise forethought through planning. Their first interest just now is to avoid wasting available funds. By establishing a long-term schedule of development on which every dollar spent will be well spent, an enormous economy is assured as compared with haphazard, uncorrelated conservation. The Plan is a device to get the people's money's worth in each phase of the work.
>
> "The people of Iowa have for twenty years dreamed of the recovery, development and wise utilization of the woods, lands and waters from which the great wealth of the state is derived. Every element of the Conservation Plan is set forth in response to a strong demand from thousands or hundreds of thousands of citizens. This report is issued, therefore, to advise on the manner in which the things they want may be crystallized into a feasible, economic program which can be actually realized.

The contents of the volume are made up of a foreword, summary of recommendations, fourteen chapters, and a bibliography. The chapters are named, respectively: I. The Genesis of the Conservation Plan; II. The Conservation Plan Survey; III. The Historical Background; IV. Iowa and Its People; V. The Conservation of Iowa's Soil; VI. The Conservation of Iowa's Waters; VII. Woodland Conservation in Iowa; VIII. The Conservation of Wild Life; IX. The Conservation of Game; X. The Conservation of Iowa's Fisheries. Resource; XI. State Preserves and State Parks; XII. The Highways; XIII. Recapitulation—Unclassified Projects; and XIV. Fulfillment of the Conservation Plan.

The sponsors of the report have rendered a distinct and valuable service in having prepared and published a readable and attractively arranged statement.

Price Indexes in China and Foreign Countries. 1932. Ministry of Industries, Nanking, China. Pp. 247.

The report, published by the Ministry of Industries of Nanking, China, records in Part I index numbers of wholesale prices; index numbers of retail prices; index numbers of cost of living. Part II has to do with foreign countries. The topics considered are the comparison of index numbers of wholesale prices in Melbourne, Tokyo, London, New York, and Paris; index numbers of wholesale prices in foreign countries; index numbers of retail prices in foreign countries; index numbers of cost of living in foreign countries.

The headings of the titles are given both in English and in Chinese.

An Annotated Bibliography of the Low Temperature Relations of Plants. By RODNEY BEECHER HARVEY. Burgess Publishing Company, Minneapolis, Minn., 1935. Pp. 223.

The author of the Bibliography is professor of plant physiology, agricultural

botany, and horticulture at the Minnesota Agricultural College and Experiment Station, University of Minnesota. He has evidently made a very thoroughgoing search of the literature on low temperature relations of plants and has rendered a signal service to the students of the subject.

The author notes that: "the first typing of part of this bibliography was done in 1927. Copies of this list were deposited with the Low Temperature Research Station at Cambridge University, with the Institute für Pflanzenkrankheiten at Bonn, with Dr. N. A. Maximov at the Institute of Applied Botany at Leningrad, and with the Office of Forage Crops Investigations of the U. S. Department of Agriculture. Students at the University of Minnesota have had free use of the citations for more than ten years. Publication of the list is now made with the hope of decreasing the labor of reference to the literature."

JACOB G. LIPMAN.

Kultura Bolot (*Marsh and Swamp Culture*). By B. D. ONOSHKO. Sel'khozgiz, Moscow, U. S. S. R., 1934. Pp. 1–574.

In this book the author reviews the results of investigations of the 32 Swamp Experiment Stations in the U. S. S. R., up to 1934. It is divided into five parts: I. The principles of agriculture on swamp soils, 9 chapters; II. Field crops for swam soils, 9 chapters; III. Meadow and pasture on swamp soils, 12 chapters; IV. Vegetable growing on swamp soils, 8 chapters; V. Miscellaneous agricultural plants on swamp soils. The book is profusely illustrated (145 plates) and has a list of close to 500 Russian references. It is to be regretted that the author failed to include at least the more important contributions of the Western European and American investigators on the subject. Even with this shortcoming it is a valuable contribution.

J. S. JOFFE.